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ANALYSIS OF FREE SUGARS AND CHLOROPHYLL IN SPINACH FROM A LOCAL RETAIL MARKET

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Harvey J. Drews

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DEDICATION

This thesis is dedicated to four of the most important people in my life:

My Wife, Harriet E. Drews, who is teaching me by her unselfish and steadfast example, the true meaning of loving, giving and caring.

My Grandmother, Florence V. Uhlmann, who with endless strength raised me by being both mother and father to me, day in and day out, and who planted her wonderful sense of humor in my memory for life.

My Mother, Eleanor E. Neilson, who provided her dedication and who sacrificed for me so that I would have a secure and love centered home.

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All of whom have provided me with the center from which I could reach forth and obtain my educational goals.

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ABSTRACT

The accuracy (percentage recovery) and precision (coefficient of variation or CV) of extraction and an anion-exchange HPLC method using amperometric detection for measurement of free sugars in spinach were determined. Five samples of fresh spinach leaves were extracted and analyzed by the HPLC method for glucose, fructose and sucrose. Different amounts (0.093 to 0.453% of spinach, wet basis or WB) of each of the latter sugars were added to each of five spinach samples and these spiked samples were analyzed in a similar manner as the unspiked samples. The average percentage recoveries of glucose, fructose and sucrose were respectively, 106.6, 101.5 and 106.0. CV's of percentage recoveries for the five spiked samples were 7.8% for glucose, 6.8% for fructose and 2.8% for sucrose.

The reported levels of total sugars in market fresh spinach in Europe range from 0.1 to 1.5% (WB) but have not been determined in the U.S.A. The recognition threshold for sweetness of sugars is approximately in the middle of the reported concentration range of sugars in spinach; thus the higher concentration ranges of sugars probably add a desirable sweetness to the fresh spinach. If levels of the sugars in fresh spinach in the U.S.A. were known, then a basis for assessment of sweetness to the flavor of such

spinach could be made. Therefore, market fresh spinach samples in Knoxville, TN, were collected from four markets weekly for an eight week period in the spring of 1995. This spinach, which is shipped in primarily from California, should be fairly representative of that consumed by Southeastern U.S. customers during that time of the year. Sugar (glucose, fructose and sucrose) levels, moisture content, and chlorophyll a and b levels were also measured. On a dry basis (DB), the fresh spinach contained 0.09 - 0.40% glucose, 0.02 - 0.20% fructose, 0.13 - 0.37% sucrose, 0.53 - 0.78% chlorophyll a and 0.18 - 0.25% chlorophyll b the fresh spinach also contained from 90.0 - 91.7% moisture. Spinach from one market had ($p<0.05$) higher levels of sucrose but lower moisture and chlorophyll contents than spinach from the other three markets. All spinach samples were dark green in color with crisp texture and were acceptable as fresh samples.

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CHAPTER I

INTRODUCTION

The analysis of minor components such as free sugars in vegetables often requires long methods for clean-up and concentration of the minor components prior to actual instrumental measurement. Martin-Villa et al. (1982) measured the free sugar levels in raw and cooked vegetables by high performance liquid chromatography (HPLC) using a refractive index detector. In their method, the latter investigators extracted the sugars with an aqueous solution and when the extracts contained colored pigments, both column chromatographic and solid phase extraction clean-up were required prior to HPLC analysis. The refractive index detector, however, is one of the least sensitive HPLC detectors available (Pomeranz and Meloan, 1987), and sample concentration also is required to determine the lower concentrations of free sugars in vegetables using this detector (Zhu et al.,1992). Preliminary work in The University of Tennessee Food Science and Technology laboratories has shown that an anion-exchange HPLC offers increased sensitivity and has more selectivity without extensive clean-up than the normal phase HPLC using a refractive index detector for the measurement of free sugars in spinach. In preliminary work, glucose, frucose and

sucrose were analyzed in alcoholic extracts of spinach at very low concentrations (.5 to 18 ppm) using anion-exchange HPLC. However, the accuracy and the precision of the method is unknown and needs to be determined.

During the spring months, fresh spinach in the markets in Knoxville, TN, is grown predominately in California and shipped in under refrigerated storage. Generally, this produce is of acceptable quality and locally is handled properly to maintain a fresh appearance. Locally, fresh spinach may be obtained in tied bunches of approximately 120 g which are in an open-air refrigerated display cases or in perforated polyethylene bags containing up to 908 g in a refrigerator. Such spinach should be representative of the spinach marketed in the eastern U.S.A., particularly in Tennessee and those states contiguous to Tennessee during that time of the year.

Spinach has one of the highest respiration rates of all fruits and vegetables, making it one of the most perishable commodities (Considine and Considine, 1982). Therefore, to slow this respiration rate during storage of produce is of utmost importance (Wills et al., 1989). Differences in fresh spinach quality may result from variability of climatic conditions under which it is grown and from improper handling and storage conditions. Optimum handling and storage conditions will slow down the respiration rate, maintain a bright, dark green color and turgor in the

spinach. Under optimum storage conditions (-1 to -5°C temperature and 90-100% relative humidity), the rate of moisture loss in spinach can be reduced significantly (Wills et al., 1989). Although the concentration of the chlorophyll pigments in fresh spinach is more dependent upon plant maturity than handling and storage conditions, mechanical injury to the leaves or improper storage resulting in significant moisture loss could cause degradation of the chlorophylls to other derivatives such as pheophytins. Schwartz et al. (1981) found that fresh spinach contained only chlorophyll a and b of the 12 chlorophylls measured. Appearance of pigments other than chlorophyll a or b or a significantly lower chlorophyll a content (>50%) from initial post harvest levels may indicate improper handling or storage of fresh spinach and may result in color changes which may cause fresh spinach to be unacceptable.

The levels of the free sugars in spinach are more dependent on the respiration rate than either moisture or chlorophyll. Reports of the total levels of the free sugars (glucose, fructose and sucrose) in fresh spinach range from 0.11 (Martin-Villa et al., 1982) to 1.5% (Holland et al., 1991). Martin-Villa et al. (1982) analyzed fresh spinach from markets in Spain while Holland et al. (1991) stated that the spinach samples analyzed were taken from shops, supermarkets and different retail outlets to be

representative of that consumed by the British population. Based on crop studies in Germany, Schuphan (1965) reported that spinach contained 0.69% total sugars. The reported lower concentration (.11%) of total sugars is below the sweet flavor recognition threshhold for sugar but the higher concentration (1.5%) is above that threshold (deMan, 1985). Therefore, the level of free sugars in fresh spinach could be important to the flavor of fresh spinach. However, no studies were found in which the levels of free sugars were measured in fresh spinach grown and marketed in the U.S.A. Assuming that the fresh spinach available at the markets in Knoxville, TN, during the spring months is fairly representative of spinach consumed by a large segment of the U.S. population, analysis of the levels of free sugars in that spinach is needed. Also, in combination with free sugar determination, the analysis of moisture content and chlorophyll pigment concentrations in the spinach would assure that the fresh spinach analyzed was of acceptable quality and had been handled and stored properly.

Therefore, the objectives of the present study were (1) to determine the accuracy and precision of an anion-exchange HPLC method for the analysis of free sugars (glucose, fructose and sucrose) in fresh cut spinach, and (2) to analyze and determine the variation in the concentrations of the free sugars (glucose, fructose, and sucrose), chlorophyll a and b and moisture in fresh cut spinach from

four retail markets in the Knoxville, TN, area during an eight week period in the spring of 1995.

CHAPTER II

LITERATURE REVIEW

Spinach Background

The commercial varieties of spinach (Spinacia oleracea) are basically classified into two groups, the savoy or wrinkled-leaf and the flat-leaf (semisavoy) types. The flat-leaf group includes such varieties as Giant Nobel and Norgreen. The Bloomsdale (long standing) and Virginia Savoy represent the wrinkled-leaf type. The wrinkled-leaf is preferred for the fresh market and the flat-leaf is used in processed spinach (Considine and Considine, 1982).

The varieties from the flat-leaf group were the only types available at the market locations in Knoxville, TN, during the time of the present survey. This bunched and packaged spinach was grown mainly in California as determined from the bag ties of the bunched spinach and on the package label.

Tennessee is also considered a significant producer of spinach, even though less than one percent of the total annual crop (Considine and Considine, 1982). According to a report by Carew as cited by (Lapedes, 1977), the economic value of the spinach crop for fresh marketing from about 10,000 acres is approximately $9,000,000. Carew goes on to say that in the U.S., the average annual farm value for

processing spinach, based on approximately 27,000 acres is about $9,000,000. The average yield obtained over a three year period in the U.S. during the mid 1970s was 5.7 tons per acre. Approximately 20% of the crop is for the fresh market, 41% for the frozen market and 39% for canning and other processing.

Spinach has a total sugar content between 0.11% (Martin-Villa et al., 1982) and 1.5% (Holland et al., 1991) on an "as received" basis. Still another investigator (Schuphan, 1965), reported levels of total sugars at 0.69% on an "as received" basis. Since the moisture content varied among investigators, their results are depicted on a "dry matter" basis (DB) in Table 1.

Table 1-Average means of the levels (%) of sugars in fresh spinach on a dry matter basis

	Percent (DB)				
Reference	Moisture	Total Sugars	Glucose	Fructose	Sucrose
Schuphan (1965)	91.01	7.73	3.78[a]		4.0
Martin-Villa et al. (1982)	88.5	0.96	0.61	0.26	0.09
Holland et al. (1991)	89.7	14.56	-	-	-

[a] Source shows glucose and fructose combined as monosaccharides.

Table 1 shows the significant differences in the average free sugars and total sugars being reported in the literature.

Analysis of sugars in spinach

Prior to the use of HPLC for quantitative analysis of individual sugars, the quantities of many sugars were difficult to accurately determine. Often quantitative results were reported as total reducing sugars which were measured collectively, and total nonreducing sugar which were determined by subtracting total reducing sugars from total sugars (Shaw, 1988). In such measurements fructose and glucose were typically the reducing sugars and sucrose the nonreducing sugar. These assumptions were generally true; however, they did not take into consideration other sugars. As examples, maltose which is a reducing disaccharide found in glucose syrups, and sorbitol, a reducing sugar alcohol, found in significant amounts in certain fresh fruits, also would be part of the total reducing sugars (Shaw, 1988).

Early procedures developed for separation of individual sugars by liquid column chromatography using ion exchange resins are considered the basis for the development of modern HPLC separation of sugars (Shaw, 1988). The method of choice for analysis of sugars has become high performance liquid chromatography (HPLC) mainly because of

the improvements in HPLC column technology which now permit picomole quantities of a variety of carbohydrates to be measured (Scott, 1992). A number of research papers have been published documenting methods using HPLC. Iverson and Bueno (1981) compared HPLC with gas liquid chromatography (GLC) for quantitative determination of sugars and reported speed and accuracy advantages of HPLC over GLC. Zhu et al. (1992) used the method of Iverson and Bueno (1981) to determine the sugars in several varieties of sweet corn and compared their results to % brix. Others who have published research on the use of HPLC to determine sugars in foods include Martin-Villa et al. (1982) who analyzed free sugars in fresh fruit and vegetables and Bolin and Huxsoll (1991) who determined sugars in lettuce stored under controlled atmosphere. Initially in The University of Tennessee Food Science and Technology lab, we followed the method used by Zhu et al. (1992) with the exceptions that a refractive index detector was used instead of an evaporative light scattering mass detector, and 20 gm samples were extracted instead of 10 gms. However, this reverse phase HPLC method was not sensitive enough for the very low levels of free sugars present in fresh spinach without time consuming cleanup and concentration steps. A more sensitive method without the need for extensive cleanup procedures was needed. An anion-exchange HPLC instrumental method using amperometric detection met this requirement. This present

study is, in part, concerned with determining the reliability of an extraction procedure and anion-exchange HPLC measurement for the determination of the free sugars, glucose, fructose and sucrose, in fresh cut spinach. The strong anion-exchange stationary phases take advantage of the weakly acidic nature of carbohydrates to give highly selective separations, while pulsed amperometric detection allows direct analysis of the non-derivativized substrates at low picamole levels (Anonymous, 1989). The extraction procedure was simplified by using the AOAC method for sugar extraction from plants (AOAC, 1984) without a frozen storage step.

Chlorophyll background

Of the three major plant pigments (chlorophyll, carotenoids and anthocyanins), chlorophyll is the most widely distributed and most important color to leafy green vegetables (Gross, 1991). All green plants contain chlorophyll a and b; in higher plant life Chlorophyll a is more prevalent than b, and both are located in subcellular organelles called plastids, specifically colored green and named chloroplasts (Gross, 1991). Higher plants contain a chlorophyll a to b ratio of 3 to 1. This ratio constitutes a parameter of the plant's physiological status, and also varies with growth conditions and environmental factors (Gross, 1991). As an example of environmental effects on the chlorophyll a to b ratio, plant species exposed to sun

have higher ratios (3.2:1 to 4:1) than shaded plants (2.6:1 to 3:1). This chemical difference in the proportional amount of chlorophyll a to b between shade plants and sun-loving plants is due to chlorophyll b having strong absorption properties in the 450-480 nm range. Chlorophyll b, therefore, captures effective light at low intensity thus filling the gap in the chlorophyll a spectrum (Gross, 1991). As to their chemical structure the molecular formula of chlorophyll a is $C_{55}H_{72}MgN_4O_5$, and of chlorophyll b is $C_{55}H_{70}MgN_4O_6$. Figure 1 shows a diagram of the molecular structure of chlorophyll a and b (Penfield and Campbell, 1991). Chlorophylls are porphyrins containing the basic tetrapyrrole ring, of which one is reduced; the four rings are coordinated with a Mg^{+2} ion. A fifth isocyclic ring is found near the third pyrrole ring. At the fourth ring the proprionic acid substituent is esterified with the diterpene alcohol phytol ($C_{20}H_{39}OH$); this is the hydrophobic part of the molecule with the rest of the molecule being hydrophylic. Chlorophyll a differs from b in that b has an aldehyde(-CHO) in place of a methyl group at position three (Gross, 1991). Pheophytins a and b are the magnesium-free derivatives of their chlorophyll counterparts; they are obtained from chlorophyll by the action of mild or dilute acid which removes the magnesium. Reports in the literature has cited the use of oxalic (Vernon, 1960) and hydrochloric acid (Lichtenthaler,

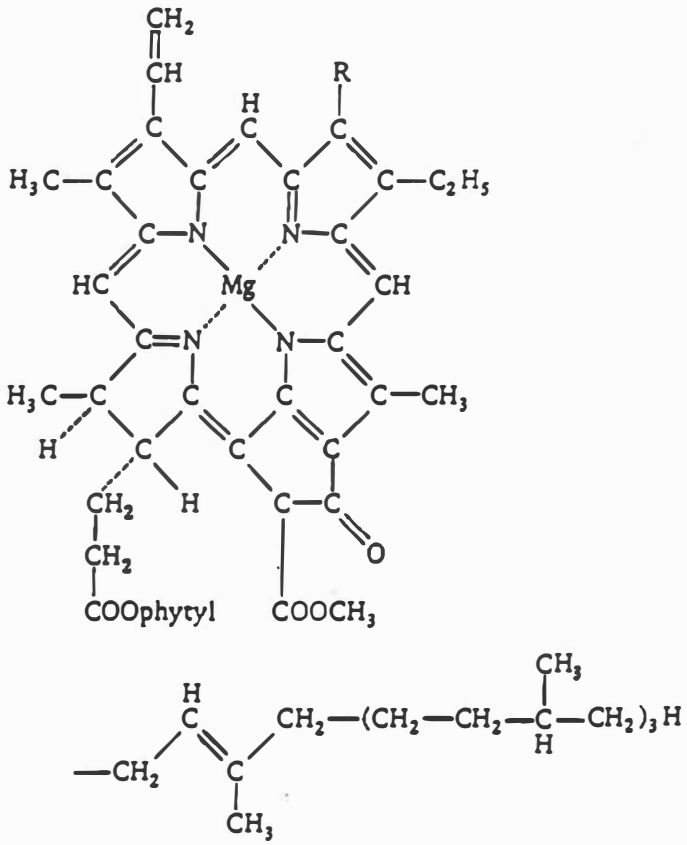


Figure 1-Chlorophyll. In chlorophyll a, R is $-CH_3$; in chlorophyll b, R is -CHO. (Penfield and Campbell, 1991)

1987) for the conversion of chlorophyll a and b to pheophytin a and b, respectively. The chlorophylls and some of their derivatives, which absorb in the blue and red regions of the visable spectrum, are green and can, therefore, be determined quantitatively by visible spectrophotometric methods.

Analysis of chlorophyll in spinach

Colorimetric methods are based upon a relationship between the concentration of a substance and its color as expressed by the Lambert-Beer Law (A=abc). The measured absorption (A), extinction, or optical density is proportional to the absorptivity (a), or specific absorption (extinction coefficient), which is known, the thickness (b) in centimeters of the sample cell and the concentration (c). The specific absorption, also referred to as $A^{1\%}_{1cm}$, or extinction, $E^{1\%}_{1cm}$ is the absorption of a 1% w/w solution in a 1 cm light path; these values are given in tables and are specific for the solvent used (Gross, 1991; Pomeranz and Meloan, 1987). The absorption is usually measured at the wavelength of the maximum absorption for the substance (Gross, 1991). Spectrophotometric determination for both chlorophylls are possible without separation into individual pigments by employing calculations using simultaneous equations in which the absorption of each chlorophyll is corrected by subtracting the contributing absorption of the other chlorophyll (Gross, 1991). A third equation for total

chlorophyll is used, in which the absorption of each chlorophyll present is added. Equations have been developed for estimating the concentration of chlorophyll and percent conversion to pheophytin in acetone extracts of vegetables (Vernon, 1960). The method involved the quantitative conversion of chlorophylls to pheophytins by acid addition and obtaining the absorbances at two wavelengths (Gross, 1991). More recently the analytical procedures for chlorophylls, pheophytins and carotenoids were reevaluated with specific emphasis on the specific absorption coefficients (Lichtenthaler, 1987). The evaluations by Lichtenthaler (1987) were carried out using a double-beam spectrophotometer, which is capable of much better resolution than the instrument used by Vernon (1960) who developed the old equations. As a result of the more recent work the former equations have given way to a new system of equations such as the following example, for a solvent mixture of 80% acetone\20% deionized water (Lichtenthaler, 1987).

$$\text{Chlorophyll a} = 12.25A_{663.5} - 2.79A_{647}$$

$$\text{Chlorophyll b} = 21.5A_{647} - 5.1A_{663.5}$$

$$\text{Chlorophyll a+b} = 7.15A_{663.5} + 18.71A_{647}$$

Where chlorophyll a = concentration of chlorophyll a in the sample, chlorophyll b = concentration of chlorophyll b in the sample, chlorophyll a+b = concentration of

chlorophyll a and b in the sample, $A_{663.5}$ = absorbance at 663.5 nm, A_{647} = absorbance at 647 nm and so forth.

The chlorophyll content in fresh spinach leaves has been reported by Gross (1991) who has cited levels determined by various workers:
576.8 µg $(g)^{-1}$ fresh weight, from Dutton et al. (1943); 1300 µg $(g)^{-1}$ fresh weight, from Kaur and Manjerkar (1975); 1576 µg $(g)^{-1}$ fresh weight, from Yamauchi et al. (1985) and on a dry matter basis 6980 µg $(g)^{-1}$ of chlorophyll a and 2490 µg $(g)^{-1}$ of chlorophyll b, from Shwartz and von Elbe (1983).

CHAPTER III

MATERIALS AND METHODS

Materials

Chemicals used in this study include the following: ACS Reagent grade glucose, fructose and sucrose (Sigma Chemical Company, St. Louis, MO); acetone, HPLC grade, (Mallinckrodt Chemical Co., Paris, KY); ethanol U.S.P. (190 proof), which was obtained from Quantum Chemical Company (Anaheim, CA), and sodium hydroxide solution (50% w/w) certified (Fisher Scientific Co., Fairlawn, NJ). Also, filters (0.2 micron, 47 mm, nylon 66) used for filtering deionized water for eluent mixture with NaOH, were manufactured by Schleicher and Schull (Keene, NH). Other material and instruments used in this present investigation are described under specific methods of analysis.

The approach for objective 1.

The approach used to determine the accuracy and precision of an anion-exchange high performance liquid chromatographic method for the analysis of free sugars in fresh spinach was as follows. Two bunches of fresh spinach were obtained from each of three retail stores in the Knoxville TN, area. The samples were held at 5-6°C, during transport to The University of Tennessee Food Science and Technology laboratories. Upon arrival, the six bunches of

fresh spinach were immediately prepared for extraction. Approximately 250 g of fresh spinach leaves were randomly selected from each of the six spinach bunches held at 5-6°C.; the stems, excess water and foreign matter were removed, leaving only the leaves. The leaves were then cut into fine pieces (approximately 0.64 cm wide and 2.54 cm long), mixed together and stored at 5-6°C until extracted.

Five portions (20 ± 2 g) of the spinach were prepared for free sugar analysis using the modified procedure of sample preparation for ethanol extraction of carbohydrates from plants in the AOAC (1984) Method 3.002(b). The modification to the extraction procedure was established by Zhu et al. (1992), in that no $CaCO_3$ was added to the extracts, and deionized water (DW) was used to establish an 80% ethanol, 20% DW (v/v) mixture.

Five, spiked spinach samples (20 ± 2 g) were prepared by adding known amounts of glucose, fructose and sucrose to each sample before extraction as described later. The spiked samples were extracted for free sugar analysis following the same procedure as previously stated for unspiked samples.

All the sample extracts were analyzed for glucose, fructose and sucrose, by the anion-exchange high performance liquid chromatographic (HPLC) procedure for carbohydrates

(Anonymous, 1989). The precision of the method was determined by calculating the standard deviation (SD) and the coefficient of variation (CV) of each free sugar for the spiked samples. The accuracy of the method was determined by calculating the average percentage recovery for each sugar in the spiked samples (Pomeranz and Meloan, 1987).

Sample Preparation and Storage

The six bunches of fresh cut spinach obtained locally for objective 1 were purchased from three different stores. Two bunches were purchased from Kroger, two from BI-LO and two from Food Lion. These samples were treated and analyzed as described previously. After HPLC analysis, the concentrations of glucose, fructose and sucrose were calculated as a percentage of spinach, wet basis (WB) as described later in each of the five samples analyzed. An average percentage (WB) for each sugar then was computed as the concentration present in the unspiked spinach.

Preparation of spiked samples and extraction of spinach samples

For spiking samples, analytical grade samples of glucose, fructose and sucrose were dried at 70°C for 24 h in a vacuum oven under reduced pressure (230 mm Hg). A known weight (approximately 9 g) of each sugar was dissolved in a single solution of 80:20, v/v, ethanol: deionized water and diluted to 100 mL. This solution was

designated as spiked sugar mixture (SSM) and five, diluted solutions were made from SSM by dilution with 80:20 v/v ethanol:deionized water (ETOH:DW) as follows:

Sample 4s : 2 mL SSM, 8 mL ETOH:DW
Sample 5s : 6 mL SSM, 4 mL ETOH:DW
Sample 6s : 4 mL SSM, 6 mL ETOH:DW
Sample 7s : 8 mL SSM, 2 mL ETOH:DW
Sample 8s : 100% SSM

These samples were used to spike spinach samples, and spinach samples were extracted as follows:

[1] A known weight (20 ± 2 g) spinach sample was spiked with 1.00 mL of sample 4s; another spinach sample of known weight (20 ± 2 g) was spiked with 1.00 mL of sample 5s; a third spinach sample (20 ±2 g) was spiked with 1.00 mL of 6s; a fourth spinach sample (20 ± 2 g) was spiked with 1.00 mL of 7s and a fifth spinach sample (20 ± 2 g) was spiked with 1.00 mL of sample 8s.

[2] Each spiked sample plus six (6.00) mL of ETOH:DW was placed in seperate 50 mL centrifuge tubes.

[3] Each of Five, (20 ±2 g) samples of spinach plus seven (7.00) mL of ETOH:DW were put into a seperate 50 mL centrifuge tube.

[4] All ten spinach samples in the centrifuge tubes were heated five min in a boiling water bath.

[5] Then, the ten samples were centrifuged for ten

minutes @ 48,200 x g, and the supernatant was decanted and retained.

[6] For each sample, the precipitate was washed with 10 mL of 80:20, v/v, ETOH:DW and recentrifuged. This procedure was repeated two additional times using 10 mL then 7 mL of the 80:20, v/v, ETOH:DW, respectively.

[7] Supernatants were combined for each sample, and each sample extracted was then diluted to 50 mL with the 80:20 v/v ETOH:DW.

[8] Ten mL of the filtered spinach extract was then placed in a rotary evaporator (70°C. and 65 rpm) and concentrated to 2-4 mL under vacuum to remove most of the ethanol, the volume was then brought up to 10 mL with deionized water.

One (1.00) milliliter of each aqueous sample solution was then diluted to 250 mL with deionized water for each sample. Then, the 10 samples each were filtered through a 0.45 µm Acrodisk filter and held at -18°C for HPLC analysis of sugars.

The Anion Exchange HPLC Sugar Analysis Equipment and Conditions

I used the procedure as outlined in the Appendix A from Dionex (1989). The anion exchange HPLC equipment

consisted of the following: A Dionex anion exchange HPLC (HPAE/PAD) system (Model DX-300); Columns, 1 Carbopac PAI (4x250 mm) and 1 Carbopac PAI (guard); a Dionex variable pulsed amperometric detector; Dionex gradient pumps; a Dionex degas module and a Dionex automated sampler (Dionex Corporation, Sunnyvale CA). The mobile phase was a HPLC grade solution of 200mM NaOH:deionized water (80:20 v/v). The flow rate was 1.00 mL per minute. The deionized water (DW) used to make up the NaOH:DW solution was filtered through a 0.45 µm millipore filter.

Preparation and analysis of sugar standard solutions

A standard solution of the sugars was made by dissolving 0.2030 g fructose, 0.3530 g glucose and 0.0520 g sucrose in and diluting to 50 mL with 80:20, v\v, ETOH:DW. This concentrated solution was used to make three dilutions for the standard curve as follows:

[1] Standard sample A, 0.1 mL concentrated solution diluted to 200 mL with deionized water.

[2] Standard sample B, 0.1 mL concentrated solution diluted to 100 mL with deionized water.

[3] Standard sample C, 1 mL concentrated solution diluted to 400 mL with deionized water.

The concentrations of each sugar in each standard sample is given in Table 2.

Table 2-Concentrations of sugars in standard samples

	Sugars		
Standard Sample	Glucose(ppm)	Fructose(ppm)	Sucrose(ppm)
A	3.53	2.03	0.52
B	7.06	4.05	1.03
C	17.66	10.13	2.58

Each standard sample was filtered as described previously and analyzed by the HPLC procedure. A standard curve relating peak area to concentration was prepared for each sugar using the regression analysis function of Statgraphics (c) version 5.0 (STSC, 1991). The standard sugar curves and regression analysis data are in the Appendix A section of this thesis.

Calculation of sugar concentrations in spinach

A constant volume (25 µL) of each sample extract was injected into the HPLC to determine the peak area of glucose, fructose and sucrose in each spinach sample. The concentration in ppm of each sugar was determined using the appropriate standard curve. The ppm was converted to mg per mL for calculations of the percent sugar in the sample of fresh spinach by the following equation:

$$\% \text{ Sugar in Spinach} = \frac{(C_x)(V_1)(V_2)(V_3)}{S} \times 100$$

Where S = spinach sample weight in mg; V_1 = first dilution volume (50 mL); V_2 = second dilution volume (25 mL); V_3 = third dilution volume (10 mL); and C_x = concentration of sugar in mg $(mL)^{-1}$. Examples are:

% Fructose in spinach

=[(.001108 mg/mL)x(50)x(25)x(10)/20000 mg]x100

=.0693%

% Glucose in spinach

=[(.001642 mg/mL)x(50)x(25)x(10)/20000 mg]x100

=.1026%

% Sucrose in spinach

=[(.000215 mg/mL)x(50)x(25)x(10)/20000 mg]x100

= .0134%

Calculations for percentage recovery

In order to determine the accuracy of the method for analysis of the sugars described previously, the pecentage recovery for each sugar was calculated using the following formula:

% Recovery = 100(y/x)

where y = the concentration of analyte detected by the test method in the spiked sample and x = the concentration of analyte calculated from the amount added or spiked plus the

average amount measured in the unspiked samples.

The average percentage recovery for each sugar was then calculated. The Grubbs test for outlier's (Taylor, 1990) was used if any of the individual percentage recoveries were suspect. A complete discription of this test is given by Taylor (1990), including the critical value tables.

Coefficient of variation

The coefficient of variation (CV) was used to determine the precision of the test method. CV was calculated by the following formula:

$$CV = [SD/(\bar{x})] x 100$$

where SD = the standard deviation of the average % recovery for each free sugar, and ($\bar{x}$) = the average mean for the % recovery data for each free sugar.

A %CV (±) 10% is generally acceptable (Melton, 1993).

The approach for objective 2

The approach to determine the concentrations of free sugars (glucose, fructose, and sucrose), chlorophyll a and b and moisture in fresh cut spinach from the retail market in the Knoxville, TN, area during an eight week period from March-May, 1995, was as follows. Two randomly selected samples (approximately 120 g each) of fresh cut spinach were obtained from the fresh produce section of each of three different retail markets and one 908 g packaged sample from

the fresh produce section of a fourth retail market in Knoxville, TN, each week, for a total of eight weeks. These markets included BI-LO (Location A), Food Lion (Location B), Kroger (Location C), and Sam's Wholesale Club (Location D).

Each week, the samples were transported in a cooler (5-6°C) to The Department of Food Science and Technology at The University of Tennessee, where the 908 g packaged sample was then divided equally into two samples. These eight samples were stored at 5-6°C at the Food Science and Technology labs no more than 4 hr while being analyzed for moisture and extracted for chlorophyll and sugar analysis.

Each of the eight spinach samples were prepared for moisture, free sugar, and chlorophyll analysis as described previously under, "The Approach for Objective 1" in Chapter 3. Sugars were extracted as previously described under this same section. The extracted sugars were stored at 5-6°C and analyzed within 24 hr after extraction by the previously described HPLC method. The concentrations of fructose, glucose and sucrose were calculated using the standard curves relating their respective HPLC peak area to concentration (ppm) described under "The Approach for Objective 1". The concentration of total sugars was also calculated for each spinach sample by adding the concentrations of the individual sugars. Concentrations of each sugar and the total sugars were then converted to

weight percentage of spinach on a dry matter (DM) basis.

Moisture of each spinach sample was determined by drying approximately 10 g samples in duplicate of the chopped fresh spinach 24 hr. at 70°C in a vacuum oven under reduced pressure (230 mm Hg) to a constant weight (AOAC, 1984). The percentage moisture was calculated for each spinach sample collected.

Chlorophyll pigments were extracted by homogenizing 6 g of each prepared spinach sample with 25 mL of acetone for one minute, at a setting of 50, in a 150-mL stainless steel homogenizing cup of a Virtis Homogenizer (The Virtis Company, Gardiner, NY) (Adsule et al., 1979; Mencarelli et al., 1988). The homogenate was covered with aluminum foil and shaken for ten minutes on a "wrist-action" Burrell shaker (Burrell Co., Pittsburgh, PA) at a speed setting of three. After being shaken, the homogenate was poured into a 50-mL centrifuge tube and centrifuged at 12,000 x g for 10 min on a Sorvall centrifuge (model # RC2-B, Sorvall Inc, Norwalk, CT). A portion (1.00 mL) of the supernatent was diluted to 50 mL with a 80:20 acetone:deionized water mixture. The visable spectrum (400-750 nm) of each extract, in % transmission, was obtained using a Shimadzu UV-160 UV-VIS recording spectrophotometer (Shimadzu Corporation, Kyoto, Japan) and saved on a floppy disk for further data processing, using PC 160 Plus personal spectroscopy software (Shimadzu Scientific

instruments Inc., Columbia, MD). The Shimadzu UV-160 UV-VIS recording spectrophotometer was used in conjunction with simultaneous equations developed by Lichtenthaler (1987) to determine the concentration of chlorophyll a and b in the spinach samples. The percentage transmission of the diluted extract of each spinach sample was determined at the wavelengths of maximum absorption as close to those used by Lichtenthaler (1987) as possible.

The percentage transmission spectrum data from each spinach sample was saved and imported via Data Interchange Format (DIF) files into a Lotus 123 (c), Version 2.01 (Lotus Development Corporation (c), 1985) spreadsheet format where the data was converted from % transmission to absorption. Using absorption at the appropriate wavelengths and the Lichtenthaler (1987) equations, the concentrations of chlorophyll a and b and total chlorophyll were calculated for each spinach sample. The concentrations of chlorophyll a and b and total chlorophyll were then converted to percentage of spinach on a dry matter (DM) basis.

Statistical design for objective (2)

The design of this experiment was factorial (4 locations types x 8 weekly sample times x 2 random sample replications) in which 64 samples were obtained (Sanders, 1994). The independent variables were the four market locations (Kroger, Sam's Wholesale Club, BI-LO and Food

Lion) and sampling times. Samples were taken on a seven day basis for eight continuous weeks during March-May, 1995. Each week, two randomly picked samples were chosen from Kroger, BI-LO and Food Lion, and one (908 g) packaged sample was randomly chosen from Sam's. The Sam's sample was divided into two halves to provide two samples for testing.

Statistical analysis

The concentration of each sugar, total sugars, each pigment, total chlorophyll and moisture were analyzed statistically as shown in Table 3. These same dependent variables were also analyzed statistically for the first half (weeks 1-4) of the collectiion period and the second half (weeks 5-8) as a function of location (LOC), week or period of sampling (WK) and LOC x WK interaction. The analysis of variance for the first and second halves of the experiment were done to show the difference in variance between these sampling periods.

The analyses of variance were done using PROC GLM option in Statistical Analysis Systems or SAS (R) (SAS Institute Inc., 1982). For the total experiment, significantly ($p<0.05$) different means among independent variables LOC and WK were separated using the PDIFF option in SAS (SAS Institute Inc., 1982). Because of the large number of degrees of freedom (21) in the AXB interaction,

Table 3-Analysis of variance[a] for sugar contents pigment concentrations and moisture levels in spinach samples obtained from local markets in Knoxville, TN, during March-May, 1995.

Source	Degree of freedom (df)
Location or store (A)	3
Week (B)	7[b]
AxB	21
Error	32
Total	63

[a]From Sanders (1994).
[b]This is based upon sampling every seventh day for eight continuous weeks.

means of each dependent variable from each location were plotted across each week of sampling to show how they differed. In addition, linear correlation coefficients among the dependent variables were obtained also using SAS (R) (SAS Institute Inc., 1982).

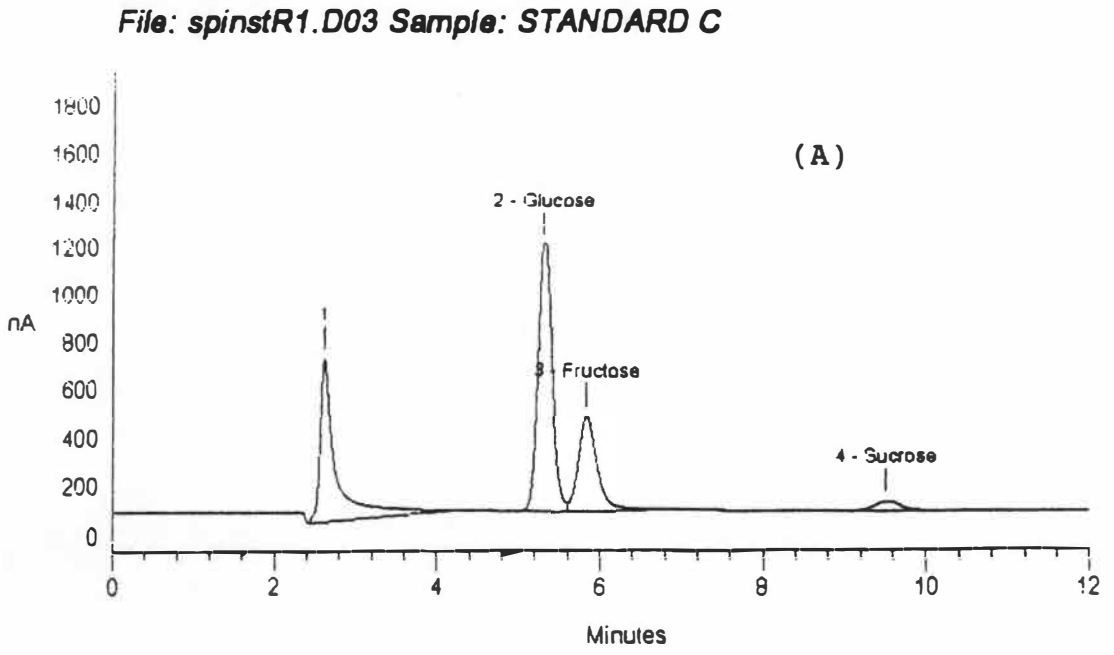
CHAPTER IV

RESULTS AND DISCUSSION

Results and discussion of the anion-exchange HPLC method for sugar analysis of spinach (objective 1)

Linear regression analyses as presented in Appendix A, Tables A-1, A-2 and A-3, were used to obtain the standard curves for glucose, fructose and sucrose respectively. The standard curves for glucose, fructose and sucrose are illustrated, respectively, in Appendix A, Figures A-1, A-2 and A-3. The glucose standard curve had a linear correlation coefficient (r) of .9982; the fructose standard curve had a r of .9990, and the sucrose standard curve, a r of .9998. The equation for each free sugar was used to calculate the concentration of that sugar in the five spiked and five unspiked spinach sugar extracts analyzed by the HPLC anion exchange method.

An HPLC chromatogram of a standard solution containing known amounts of glucose, fructose and sucrose is presented in Figure 2, part (A). The HPLC chromatogram of a sugar extract from a spinach sample also is shown in Figure 2, part (B). The retention times for glucose, fructose and sucrose in the HPLC anion exchange method were, respectively, 5.30, 5.83 and 9.47 min (Appendix A, Table A-4). The peak area and corresponding concentration of



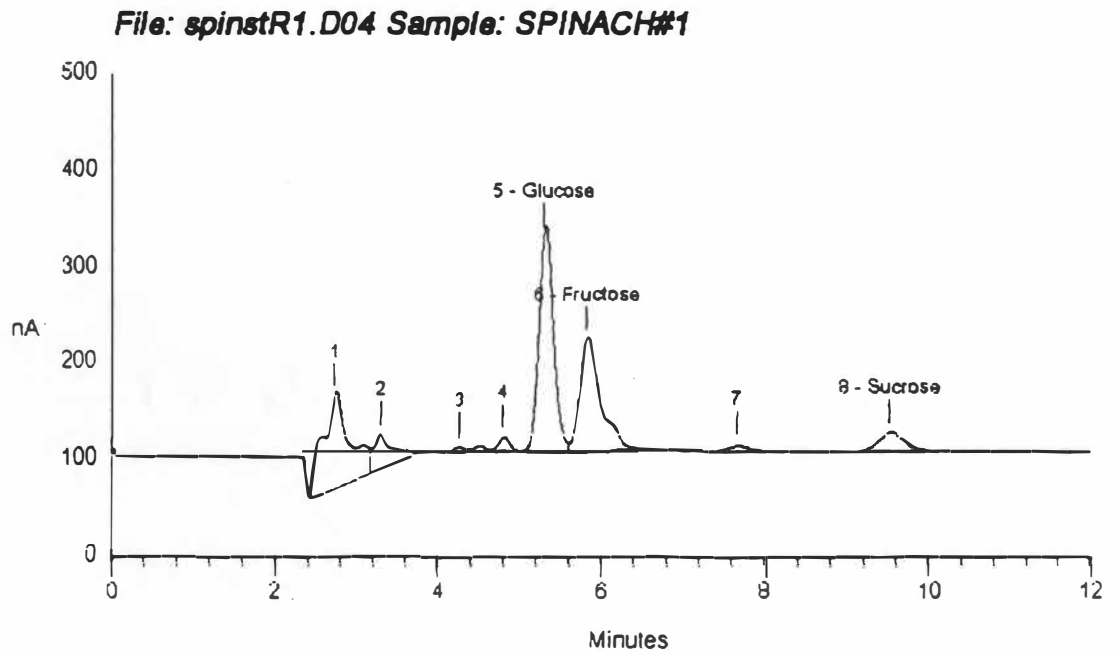


Figure 2-Examples of two anion exchange HPLC chromatograms, (A) is a standard sugar sample and (B) is a spinach sugar extract sample.

each sugar in the standard sugar mixture are shown in Appendix A, Table A-5. Actual peak area and corresponding concentration of each sugar measured in unspiked and spiked samples are presented in Appendix A, Table A-6. The percentages by weight of glucose, fructose, and sucrose measured by HPLC analyses in spiked spinach samples, the calculated amount of each sugar in the spiked samples and the percentage recovery are listed in Tables 4, 5 and 6, respectively.

Percentage Recoveries

The mean percentage recovery for glucose was 106.6 ± 8.3%, for fructose, 101.5 ± 6.9% and for sucrose, 106 ± 3%. In the calculation for recovery of sucrose one sample (6s) was determined to be an outlier as described previously (Table 6). The method of analysis and the quantitation by anion exchange HPLC can be considered accurate for each sugar since the percentage recovery is 100 ± 10% (Melton, 1993).

The coefficient of variation

As stated earlier, the coefficient of variation (CV) is a measurement of precision of a test method. The CVs for the sugars analyzed by the test method were as follows:

$$CV_{(GLUCOSE)} = 7.8\%$$

$$CV_{(FRUCTOSE)} = 6.8\%$$

$$CV_{(SUCROSE)} = 2.8\%$$

Table 4-Concentration[a] of glucose in spiked spinach samples from HPLC analyses and from calculation of amount added to an unspiked spinach sample and the percent recovery.

Sample number	Glucose (%) added	Glucose (%) HPLC[b]	Glucose (%) calc[c]	Recovery (%)
4s	.0927	.2182	.2145	101.7
5s	.2522	.4220	.3740	112.8
6s	.1755	.3506	.2973	117.9
7s	.3703	.4856	.4921	98.7
8s	.4527	.5848	.5745	101.8

[a]Percent by weight of "as received" spinach sample.
[b]Concentration calculated from HPLC analysis.
[c]Concentration added + 0.1218% calculated from HPLC analysis in unspiked spinach samples.

Table 5-Concentration[a] of fructose in spiked spinach samples from HPLC analyses and from calculation of amount added to an unspiked spinach sample and the percent recovery.

Sample number	Fructose (%) added	Fructose (%) HPLC[b]	Fructose (%) calc[c]	Recovery (%)
4s	.0927	.2378	.2457	96.8
5s	.2522	.4264	.4052	105.2
6s	.1755	.3659	.3285	111.4
7s	.3703	.4935	.5233	94.3
8s	.4527	.6041	.6057	99.7

[a]Percent by weight of "as received" spinach sample.
[b]Concentration calculated from HPLC analysis.
[c]Concentration added + 0.1530% calculated from HPLC analysis in unspiked spinach samples.

Table 6-Concentration[a] of sucrose in spiked spinach samples from HPLC analyses and from calculation of amount added to an unspiked spinach sample and the percent recovery.

Sample number	Sucrose (%) added	Sucrose (%) HPLC[b]	Sucrose (%) calc[c]	Recovery (%)
4s	.0927	.1449	.1381	104.9
5s	.2522	.3264	.2976	109.7
6s	.1755	.2969	.2209	134.4[d]
7s	.3703	.4270	.4157	102.7
8s	.4527	.5311	.4981	106.6

[a]Percent by weight of "as received" spinach sample.
[b]Concentration calculated from HPLC analysis.
[c]Concentration added + 0.0454% calculated from HPLC analysis in unspiked spinach samples.
[d]This result was found to be an outlier by The Grubbs Test, and was omitted from the % recovery calculations.

A method can be considered fairly precise if the CV is ± 10% (Melton, 1993). Therefore, the analysis method tested in the present study is precise enough to be used for the quantification of glucose, fructose and sucrose in spinach samples.

The fresh spinach survey (objective 2)

Due to the changes in moisture content of spinach that can occur during sampling and storage, all sugar and chlorophyll contents were calculated on a dry matter basis (DB) weight percentage of spinach. Moisture levels were reported as percentage of spinach but on a wet matter basis (WB). The calculations were done using Lotus 123 (c) Version 2.01 (Lotus Corporation, 1985) software.

Sugar concentrations

The levels of glucose, fructose and total sugars in spinach samples collected weekly during an eight week period March-April, 1995, from four different locations in Knoxville, TN, were not affected ($p>0.05$) by location but were different ($p<0.05$) from week to week (Appendix B; Tables B-1, B-2 and B-4). Sucrose concentrations in the spinach samples were different among locations ($p<0.05$) (Appendix B, Table B-3). The interaction of location by sampling time (week) approached significance ($p<0.0517$) at the 5% level for sucrose. Although the interaction of location by sampling time was not significant for glucose,

fructose or total sugars at $p<0.05$, the levels for any one of these components from each of the four locations are plotted across sampling time to show the complexity of the data obtained (Figures 3, 4 and 5). The concentrations of sucrose for each location are also plotted across sampling time (Figure 6).

Least square mean concentrations of glucose, fructose, sucrose and total sugars averaged across sampling times for each location are shown in Table 7. Spinach from Food Lion and Kroger had the highest levels of sucrose while spinach from Sam's had the lowest level.

Least square mean concentrations of the sugars averaged across location for each sampling time (week) are presented in Table 8. The concentration of each sugar was significantly different among sample times. The highest levels of the free sugars and thus, total sugars, were generally found in spinach samples obtained during week 1 and then from week 4 through week 8. The lowest concentration of the sugars occurred generally during week 2. Generally, for any one sugar greater variation existed in the sugar concentratons among sampling times (WK) in Weeks 1-4 than in Weeks 5-8 (Appendix B, Tables B-1 through B-4). As can be seen in Figures 4-7, the concentration of any one sugar or total sugars had fairly wide ranges of concentrations for locations in any one sampling period and across the eight weeks of sampling. In spinach samples

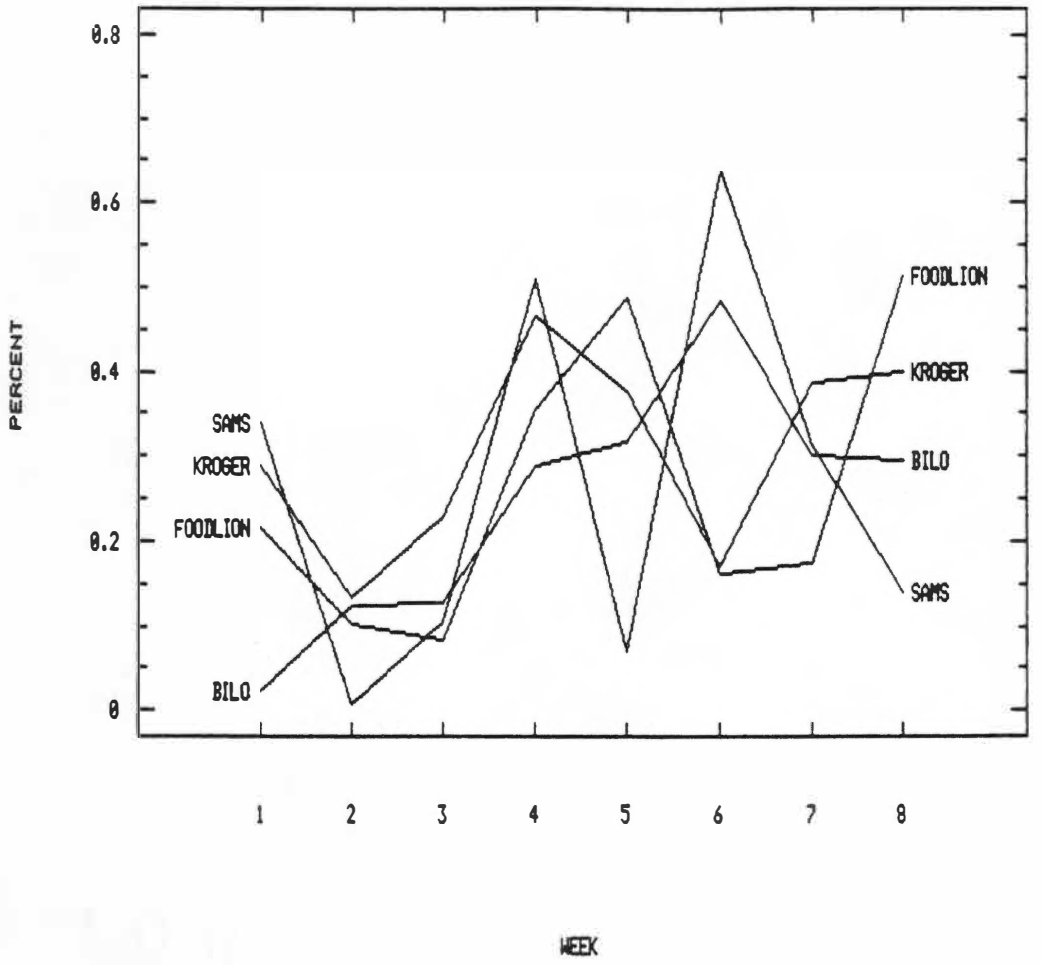


Figure 3-Glucose concentrations (% spinach, dry matter basis) in spinach samples collected from four locations (BI-LO, Food Lion, Kroger and Sam's) weekly during eight weeks in the spring of 1995.

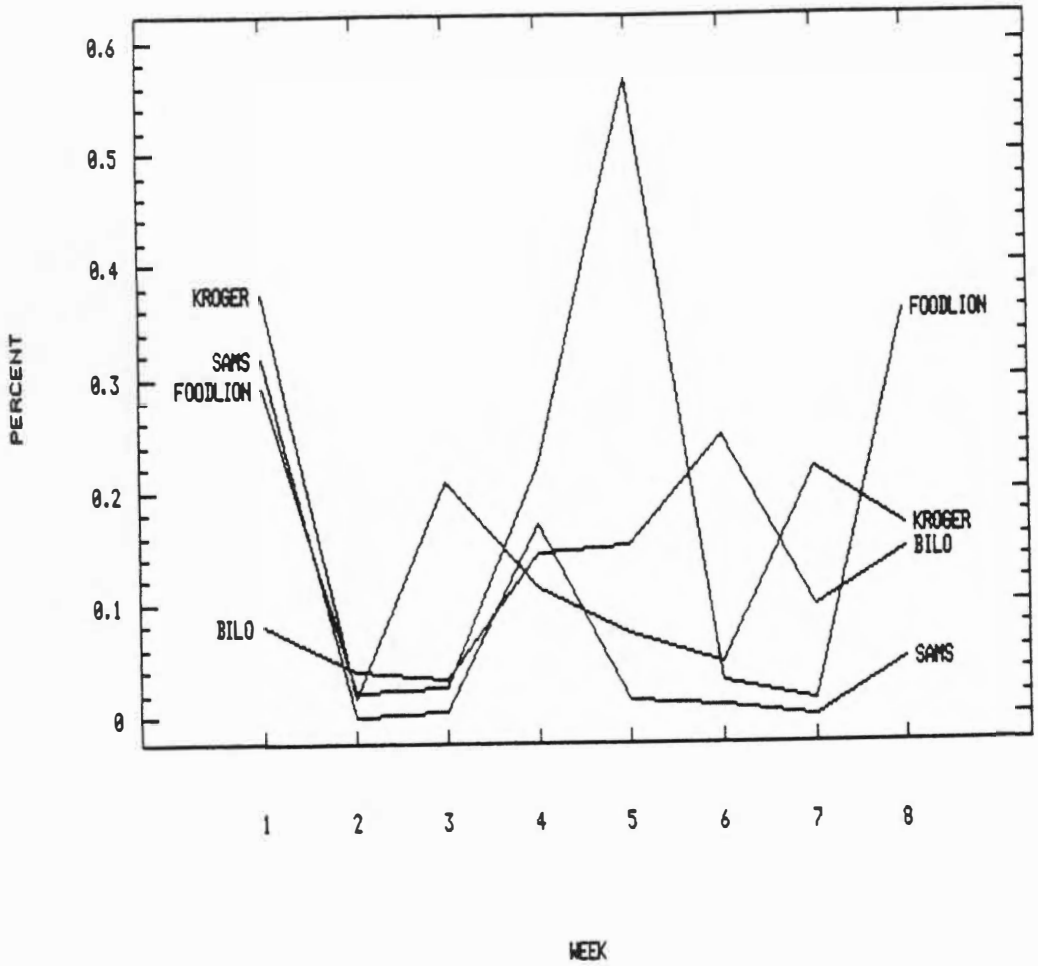


Figure 4-Fructose concentrations (% spinach, dry matter basis) in spinach samples collected from four locations (BI-LO, Food Lion, Kroger and Sam's) weekly during eight weeks in the spring of 1995.

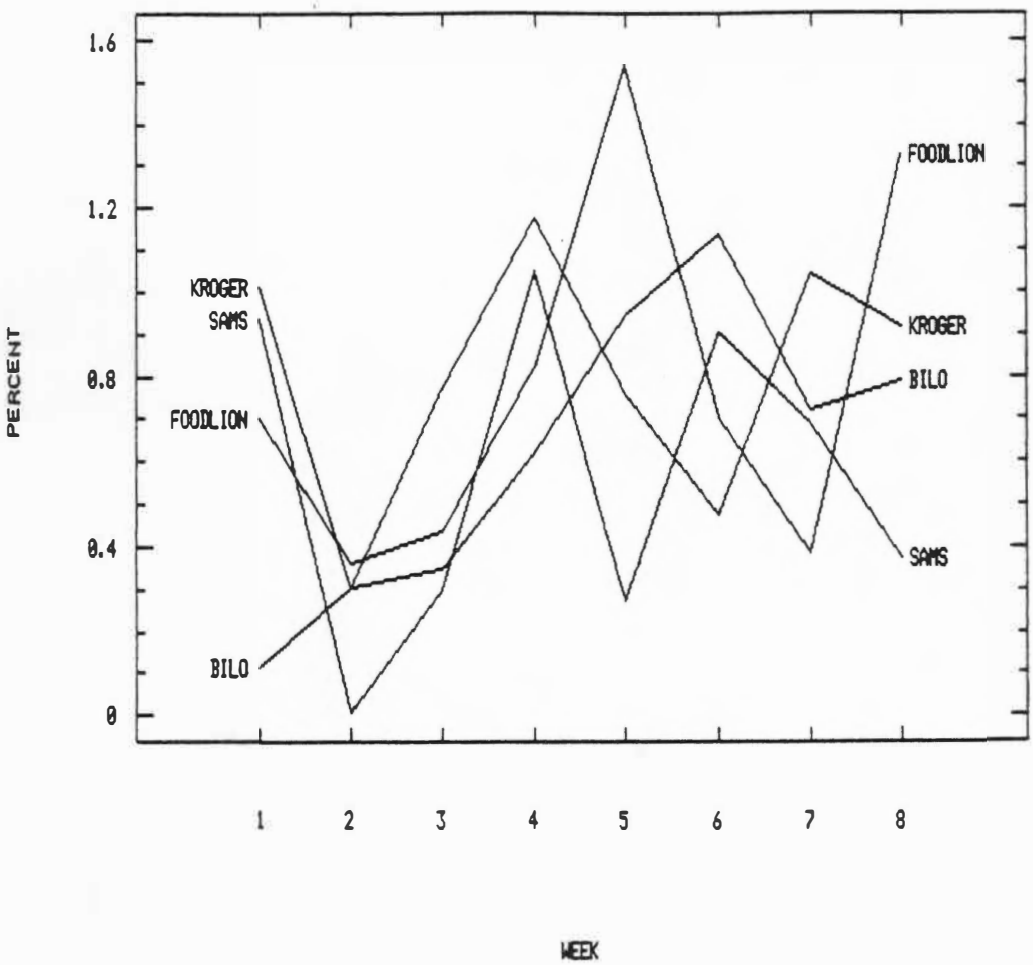


Figure 5-Total sugar concentrations (% spinach, dry matter basis) in spinach samples collected from four locations (BI-LO, Food Lion, Kroger and Sam's) weekly during eight weeks in the spring of 1995.

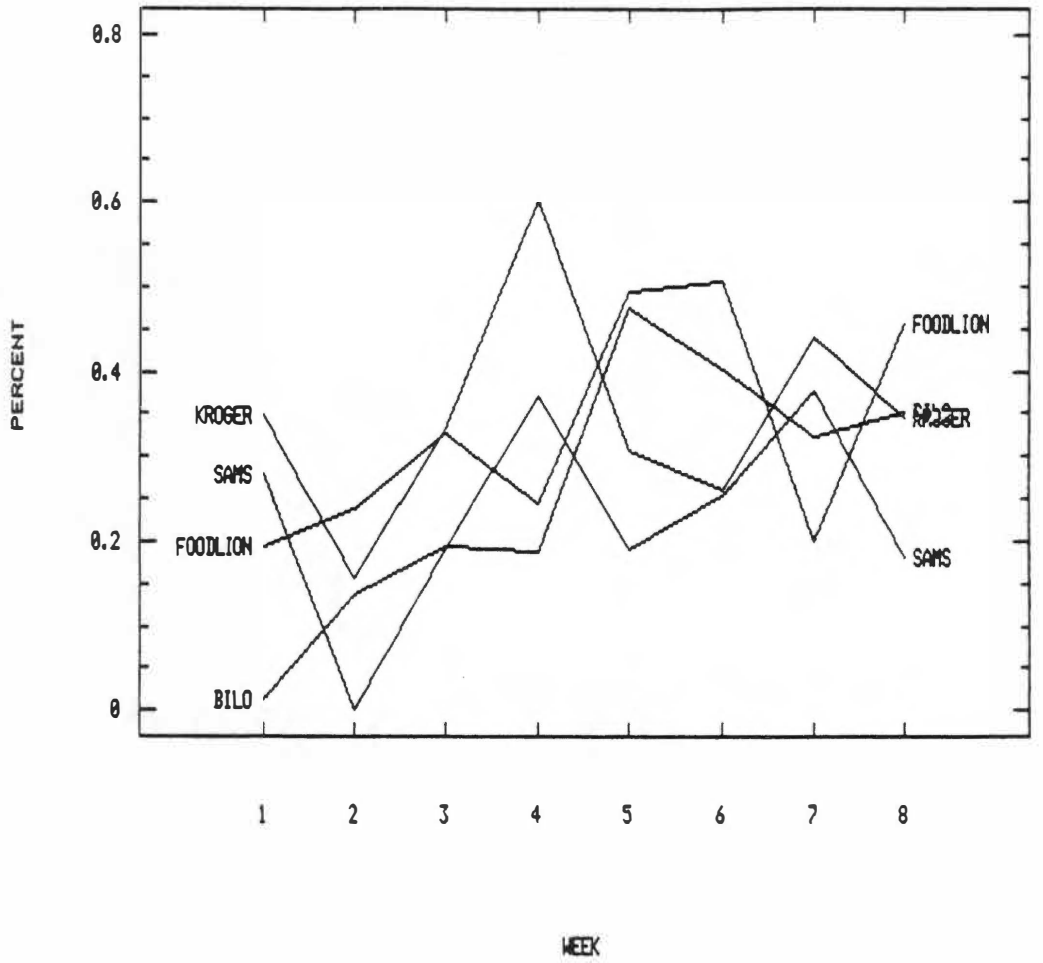


Figure 6-Sucrose concentrations (% spinach, dry matter basis) in spinach samples collected from four locations (BI-LO, Food Lion, Kroger and Sam's) weekly during eight weeks in the spring of 1995.

Table 7-Least squares means concentrations[a,b] of sugars, chlorophylls and moisture in spinach samples collected from four different locations in Knoxville, TN, during an eight week period in the spring of 1995

	Location			
Component	A	B	C	D
Glucose[c]	0.24	0.26	0.31	0.27
Fructose[c]	0.12	0.19	0.15	0.07
Sucrose[c]	0.26ab	0.33a	0.34a	0.23b
Total Sugar[c]	0.62	0.78	0.81	0.57
Chlorophyll a[d,e]	0.68a	0.67a	0.59b	0.65a
Chlorophyll b[d,e]	0.23a	0.24a	0.20b	0.23a
Total Chlorophyll[d,e]	0.91a	0.91a	0.79b	0.88a
Moisture[d,f]	91.57a	91.13b	90.56c	91.35ab

[a]Percentage spinach, dry basis, except for moisture which is on a wet basis.
[b]Means in a row followed by unlike letters are different ($p<0.05$).
[c]N=16 except for location B where N=15.
[d]Also had a location by sampling time (week) interaction ($p<0.05$).
[e]N=15 for location A and location B, and N=16 for location C and location D.
[f]N=16.

Table 8- Least square mean concentrations[ab] of sugars, chlorophylls and moisture in spinach samples collected weekly for eight weeks from locations in Knoxville, TN, in the spring of 1995

	Week							
Component	1	2	3	4	5	6	7	8
Glucose[c]	0.22abc	0.09c	0.14bc	0.40a	0.31ab	0.36a	0.29ab	0.34a
Fructose[c]	0.27a	0.02c	0.07bc	0.16abc	0.20ab	0.09abc	0.08bc	0.18ab
Sucrose[c]	0.21abc	0.13c	0.26ab	0.35a	0.37a	0.35a	0.35ab	0.33ab
Total sugars[c]	0.69ab	0.24c	0.46bc	0.92a	0.88ab	0.80ab	0.71ab	0.85ab
Chlorophyll a[de]	0.78a	0.75a	0.66b	0.53c	0.55c	0.71ab	0.63c	0.56c
Chlorophyll b[de]	0.29a	0.27ab	0.21de	0.21de	0.20de	0.25bc	0.22cd	0.18e
Total chlorophyll[de]	1.07a	1.02a	0.87bc	0.74cd	0.75cd	0.96ab	0.85bcd	0.73d
Moisture[df]	91.37a	91.68a	91.20ab	91.37a	90.01c	91.65a	91.26a	90.67b

[a]Percentage of spinach, dry basis, except for moisture which is percentage spinach, wet basis.
[b]Means in a row followed by unlike letters are different ($p<0.05$).
[c]N=8 except for week 5 where N=7.
[d]Has a location x sampling time (week) interaction ($p<0.05$).
[e]N=8 except for week 1 where N=6.
[f]N=8.

analyzed in this study, the concentration of glucose ranged from .005 to 1.17% (DB), of fructose from 0.00 to 0.71% (DB), of sucrose from 0.00 to 0.79% (DB), and of total sugars, 0.005-1.71% (Appendix B, Table B-10). The mean concentration (N=63) of glucose was 0.27 ± 21% (DB); of fructose, 0.13 ± 0.16% (DB); of sucrose, 0.29 ± 0.16% (DB); and of total sugars, 0.68 ± 0.45% (DB).

The average concentrations of the sugars are of the same order of magnitude as the levels on a dry basis reported by Martin Villa et al. (1982) in fresh spinach obtained in Spain. These latter researchers found concentrations of sugars expressed as % spinach (DB) to be as follows: glucose, 0.61%; fructose, 0.26%; sucrose, 0.09% and total sugars, 0.96%. The order of preponderance for free sugars found by Martin- Villa et al. (1982) (glucose, fructose and sucrose) are in disagreement, however, with the order (sucrose, glucose, and fructose) found in the present study. However, Schuphan (1965) found slightly higher levels of sucrose than glucose in spinach taken directly from farm plots, but the levels were an order of magnitude larger than concentrations found in the present study.

The chlorophyll a and b levels in spinach

Figure 7 represents a typical spectra of spinach extract as seen throughout the present survey.

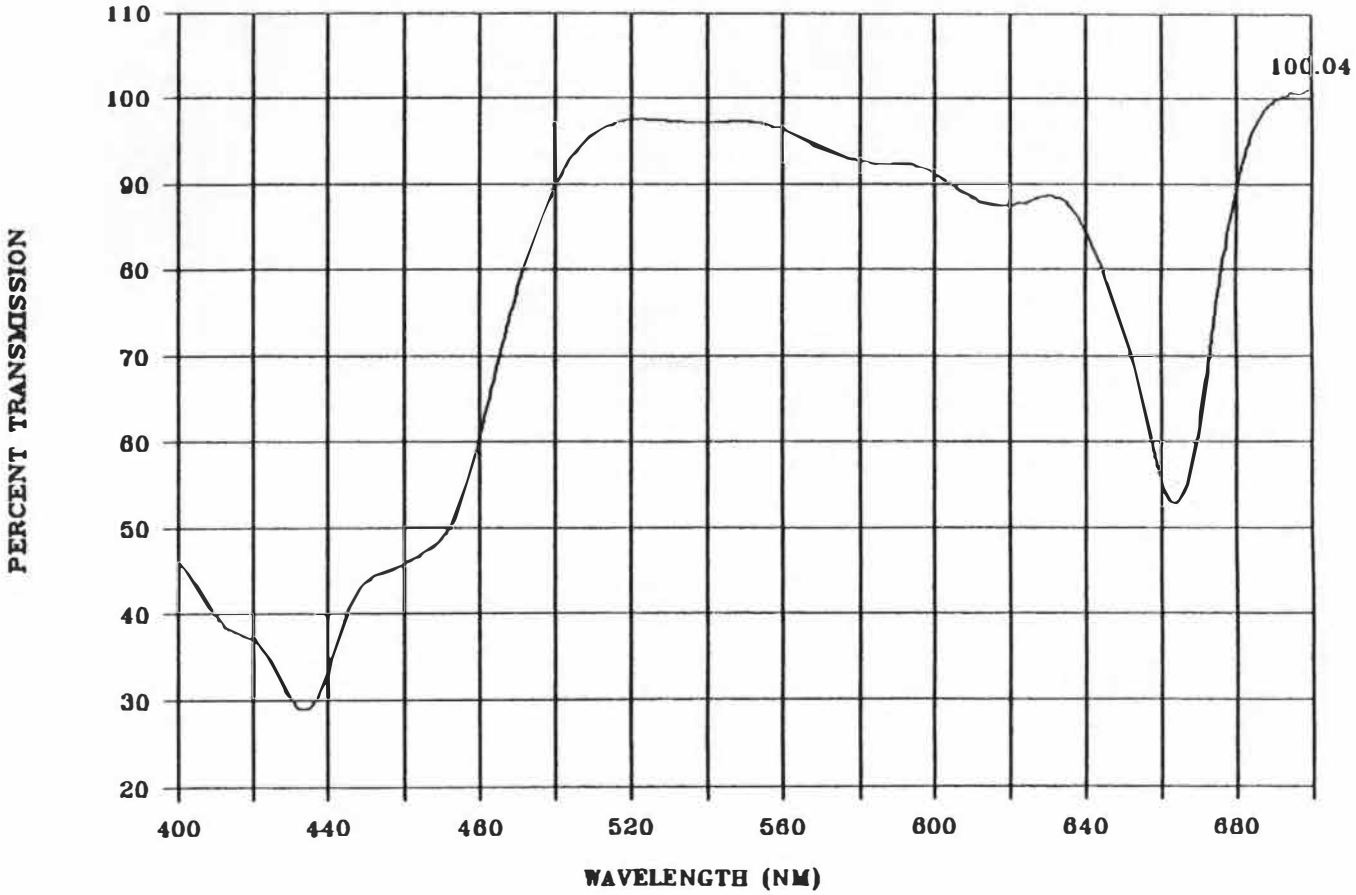


Figure 7-Percentage transmission spectrum of a spinach pigment extract, in visable wavelength range.

Concentrations of chlorophyll a and b and total chlorophyll were affected ($p<0.05$) by location, sampling period and their interaction. In general, spinach obtained from Kroger had the lowest levels of the chlorophyll pigments of all locations sampled (Table 7). However, the concentrations of the individual chlorophylls or of total chlorophyll in spinach from the different location were dependent on the week in which they were collected as shown in figures 8-10. The pattern shown in these latter figures shows the many significant interactions of locations x week of sampling. Minimum levels of chlorophyll pigments were found generally in spinach samples collected during weeks 4, 5 and 8 (Table 8), but the levels were dependent also upon the location during those weeks.

There was greater variation in pigment concentrations among locations (LOC) and weeks (WK) during sampling weeks 1-4 of sample collection than during weeks 5-8 (Appendix B, Tables B-5 through B-7). This greater variation was due to a wider concentration difference among locations and among weeks 1-4 as shown by the larger mean squares for those sources during that same period. The variation between replications, however, was less during weeks 1-4 of sample collection than during weeks 5-8 (Appendix B, Table B-5 through B-7). Spinach collected during this experiment contain from 0.21 to 1.03% (DB) chlorophyll a and 0.12 to 0.38% (DB) chlorophyll b. These average concentrations are

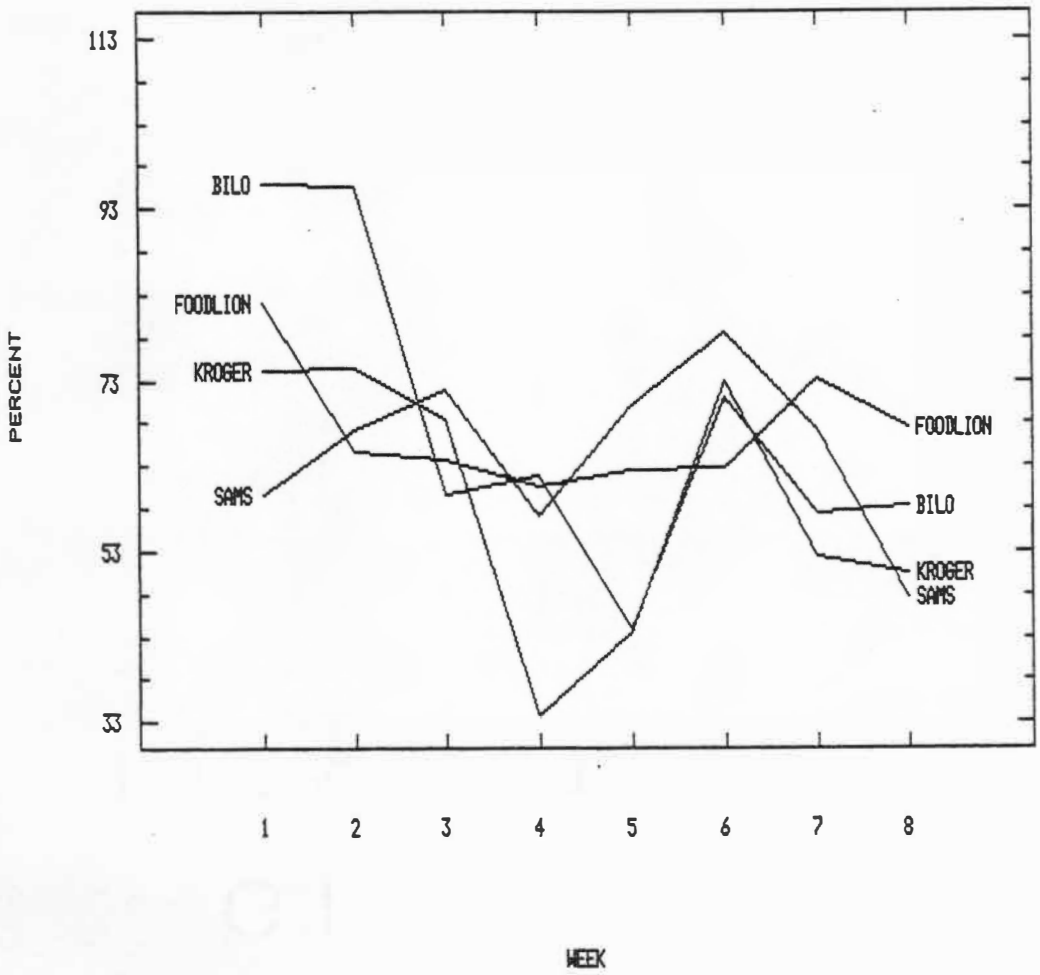


Figure 8-Chlorophyll a concentrations (% spinach, dry matter basis) in spinach samples collected from four locations (BI-LO, Food Lion, Kroger and Sam's) weekly during eight weeks in the spring of 1995.

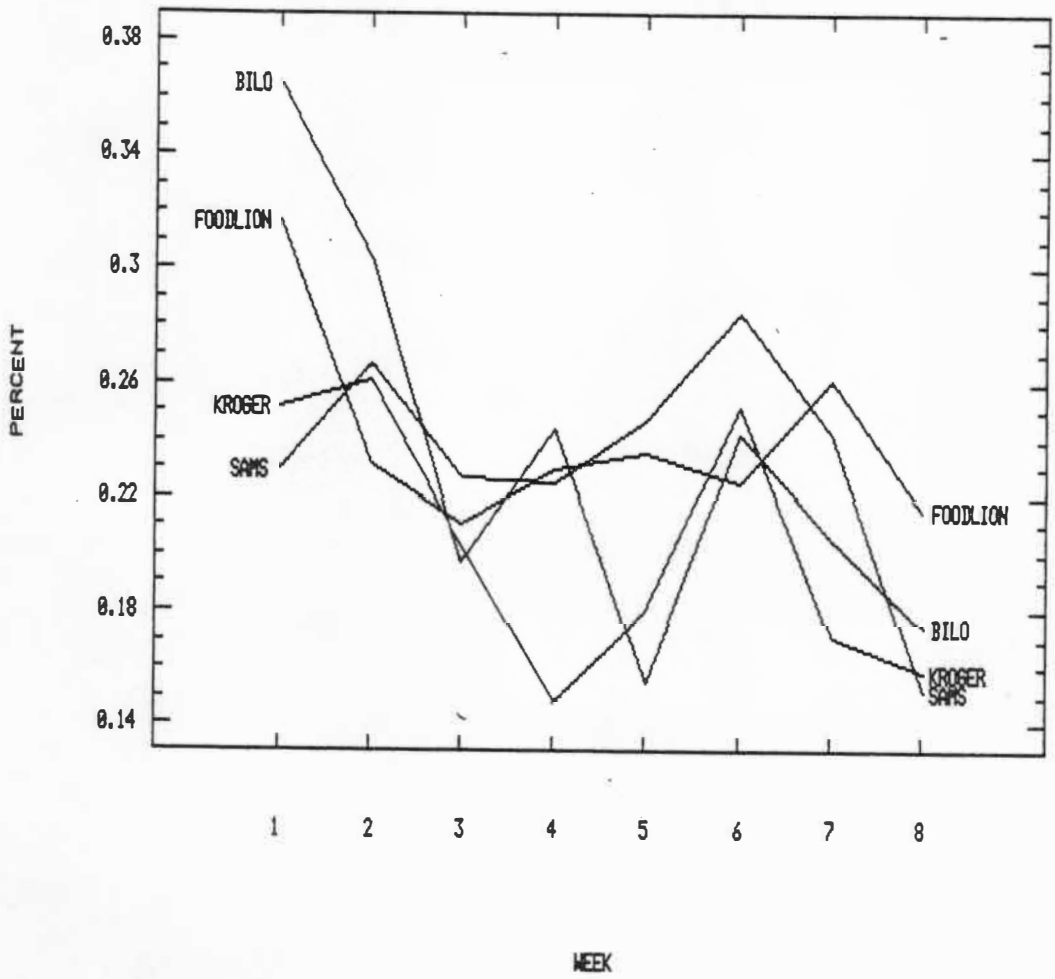


Figure 9-Chlorophyll b concentrations (% spinach, dry matter basis) in spinach samples collected from four locations (BI-LO, Food Lion, Kroger and Sam's) weekly during eight weeks in the spring of 1995.

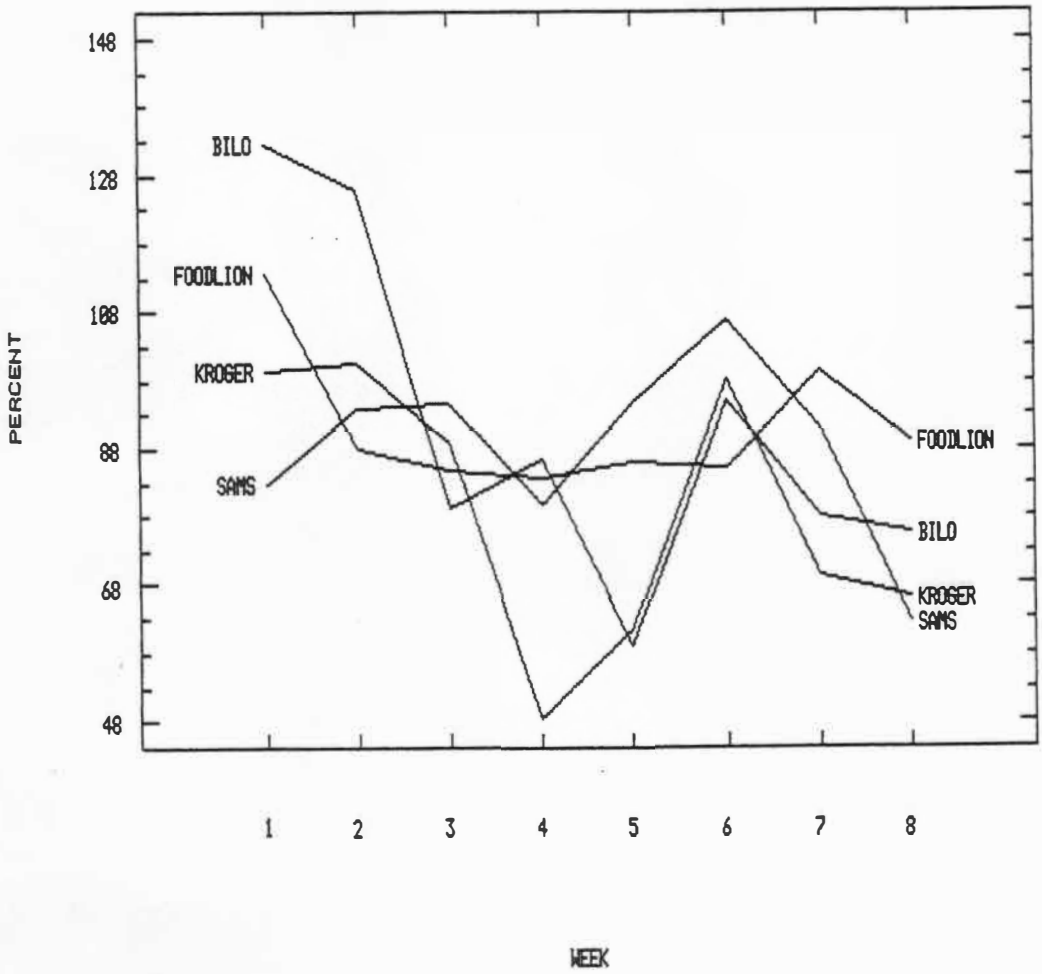


Figure 10-Chlorophyll a+b concentrations (% spinach, dry matter basis) in spinach samples collected from four locations (BI-LO, Food Lion, Kroger and Sam's) weekly during eight weeks in the spring of 1995.

in close agreement with levels of chlorophyll a (0.70%, DB) and chlorophyll b (0.25%, DB) found in spinach by Schwartz and von Elbe (1983).

Moisture level in spinach

The moisture content of the spinach also was affected ($p<0.05$) by location, week of sampling and their interaction (Appendix B, Table-8). Spinach collected from Kroger had the lowest level of moisture among all four locations sampled (Table 7). Spinach from BI-LO had higher moisture content than did spinach from Food Lion, and spinach from Sam's had moisture concentration intermediate between spinach from BI-LO and Food Lion (Table 7). Spinach samples collected during week 5 had the lowest level of moisture while spinach samples collected during weeks 1, 2, 4, 6 and 7 contained the highest levels of moisture (Table 8). However, the level of moisture in spinach from any one location was also dependent upon week of sampling as shown in Figure 11.

Moisture level in spinach analyzed in this study ranged from 88.94 to 92.63% with an average (N=64) of 91.15 ± 0.88%. Compared with samples collected from different locations during weeks 5-8, samples collected from different locations during weeks 1-4 had greater variation as shown by a larger mean square for location (LOC) in Appendix B, Table B-8. There was also less difference between replications during weeks 1-4 than during weeks 5

-8 as shown by a 10 fold plus larger error mean square given in this same table.

The average moisture concentration found in the present study (91.15%) agrees with the concentration of moisture (91.01%) reported in freshly harvested spinach from the farm by Schuphan (1965). The lower limit of the moisture concentration range measured in this study is more in agreement with moisture levels found in fresh spinach obtained from markets in Spain (88.5%) by Martin-Villa et al., (1982) and from spinach obtained from different retail outlets in Great Britian (89.7%) by Holland et al. (1991). It is apparent that storage conditions for fresh spinach at all four locations sampled were adequate in the maintenance of moisture in the fresh spinach. However, since moisture level in fresh spinach for Kroger was less than that in spinach from the other three locations (Table 7 and Figure 11), it is not unreasonable to assume that Kroger had a lower relative humidity surrouding the fresh produce than the other three locations.

Correlation coefficients among all independent variables, each sugar concentration, total sugar level, concentration of chlorophyll a, chlorophyll b and total chlorophyll, and moisture content of the spinach samples are given in Appendix B, Table B-9. High positive correlation coefficients ($r \geq 0.50$; $N=63$; $p<0.0001$) were found among

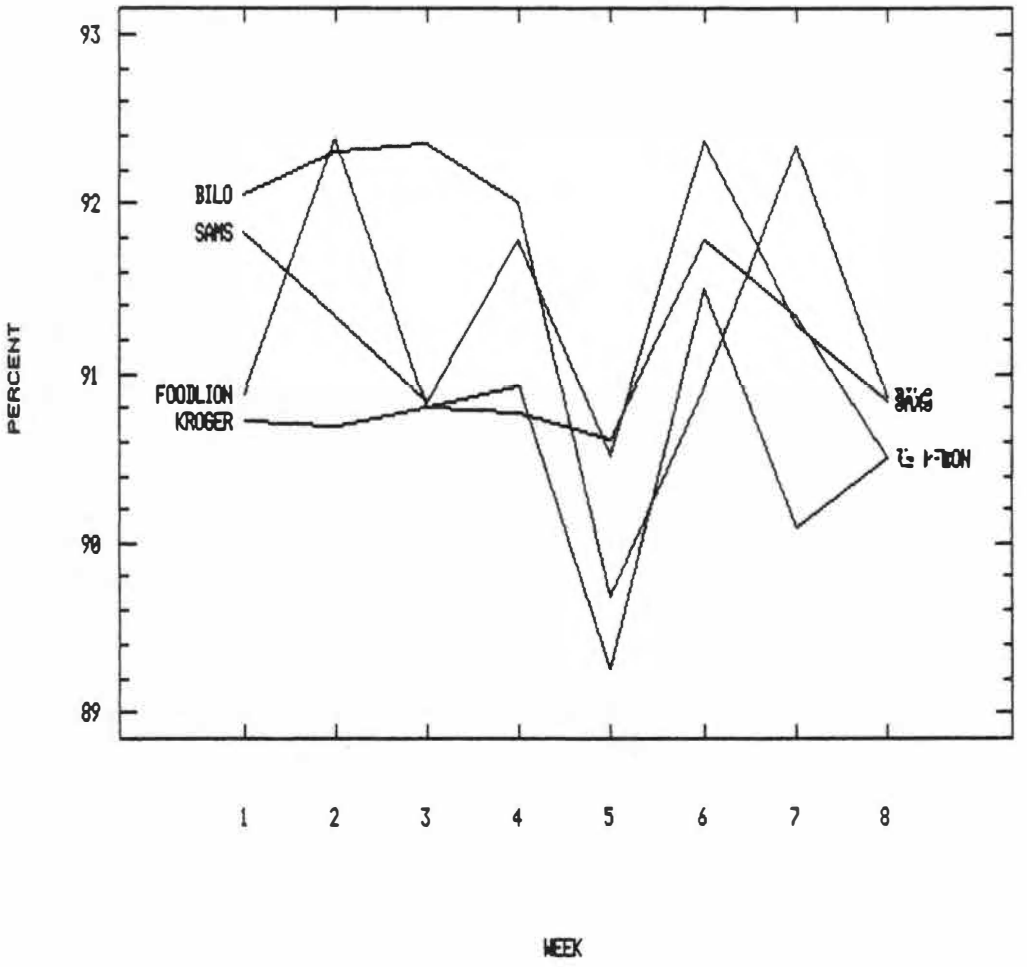


Figure 11-Moisture concentrations (% spinach, wet matter basis) in spinach samples collected from four locations (BI-LO, Food Lion, Kroger, and Sam's) weekly during eight weeks in the spring of 1995.

levels of all individual free sugars and total sugars and among the concentration of individual chlorophylls and total chlorophyll ($r \geq 0.96$; $N=62$; $p<0.0001$). Sucrose level was negatively correlated with concentrations of chlorophyll a ($r=-0.42$; $N=61$; $p<0.0007$), chlorophyll b ($r=-0.47$; $N=61$; $p<0.0001$) and total chlorophyll ($r=-0.44$; $N=61$; $p<0.0004$). A possible reason for the inverse correlation between sucrose and the chlorophylls may be the fact that sucrose is a storage reserve for glucose. Sucrose is converted to starch first, which, in turn is converted to glucose for respiration (Wills et al., 1989). Also, the higher the chlorophyll content, the greater the chance for increased respiration in green vegetables.

Compared with spinach from the other locations in this study, the lower levels of chlorophyll and moisture in fresh spinach obtained from Kroger may indicate a longer time period between harvest and retail display or of storage in an atmosphere with lower relative humidity. However, lower levels of chlorophyll and moisture in Kroger spinach did not result in noticeable loss of green color or in loss of turgor in the fresh spinach. Spinach samples collected from all locations in this study generally appeared very acceptable.

The low levels of free sugars in spinach in the Knoxville, TN. area may be inherent to the growing, harvesting and marketing practices of fresh spinach in the

United States. However, it is possible that different spinach cultivars containing higher levels of free sugars than the cultivars in the United States were analyzed by Schuphan (1965) in Germany and by Holland et al., (1991) in Great Britian. More research is needed to establish effects of different cultivars and of different storage and marketing practices on the concentration of minor components in fresh spinach. It also would be desirable to determine if differences in concentrations of minor components affect the fresh spinach likability.

CHAPTER V

SUMMARY

There were two objectives in this study. The first, was to determine the accuracy and precision of an extraction and anion exchange high performance liquid chromatographic (HPLC) method for determining free sugars in spinach. The second objective was to analyze the concentrations of free sugars, chlorophylls and moisture in fresh spinach from markets in the Knoxville, TN, area during eight weeks of the spring of 1995.

The test method used for extraction and anion-exchange HPLC analysis of sugars in fresh spinach was found to be both accurate and precise enough to be used for the analysis of glucose, fructose and sucrose in fresh spinach samples. The mean percentage recoveries for glucose, fructose, and sucrose were, respectively, 106.6, 101.5, and 106.0%. The coefficients of variation across five samples were for glucose, 7.8%; fructose, 6.8%; and sucrose, 2.8%.

During March-May 1995, two fresh spinach samples were collected from each of four different markets in Knoxville, TN, weekly for a period of eight consecutive weeks. These samples were analyzed for concentrations of free sugars (glucose, fructose, sucrose and total sugars), chlorophyll pigments (a, b and total) and moisture.

The fresh spinach contained an average of 0.27 ± 0.21% (dry basis or DB) glucose, 0.13 ± 0.16% (DB) fructose, 0.29 ± 0.16% (DB) sucrose and 0.68 ± .45% (DB) total sugars. However, the concentrations of each sugar and total sugars differed ($p<0.05$) from week to week during the sampling period. For example, spinach samples contained a concentration range of from 0.005 to 1.17% (DB) glucose, 0.00 to 0.71% (DB) fructose, and from 0.00 to .79% (DB) sucrose. The only sugar that differed significantly from one location to another was sucrose; spinach from one location had less sucrose than spinach from two of the other locations.

The levels of chlorophyll a and b and of total chlorophylls in the fresh spinach were different ($p<0.05$) among locations and from week to week of sample collection. The manner in which the level of any one pigment differed across weeks, however, was dependent upon the location as shown by a significant location x sampling time (week) interaction. Overall, spinach from one location had lower levels of the chlorophyll pigments than did spinach from the other three locations. Fresh spinach contained an average of 0.64 ± 0.29% (DB) chlorophyll a and 0.23 ± 0.05% (DB) chlorophyll b. In the spinach samples, chlorophyll a concentration ranged from 0.21 to 1.03% (DB) and chlorophyll b, from 0.12 to 0.38% (DB).

Moisture level in the spinach samples also differed

($p<0.05$) among locations and weeks of sampling, and the manner in which moisture level changed among weeks of the sampling was different from one location to another. Spinach from one location contained less moisture than spinach from the other three locations. Spinach contained an average (N=64) of 91.15 ± .88% moisture which ranged from 88.94 to 92.63% in the spinach samples analyzed.

In general, the mean concentration of the total free sugars (0.68%, DB) in spinach in this study was closer to the lowest end (0.96%, DB) of the concentration range of total free sugars reported in fresh market spinach (Martin-Villa et al., 1982). Also chlorophyll levels were similar to those reported by other researchers (Schwartz and von Elbe (1983). However, the mean moisture level in spinach in this study was closer to the level reported in spinach fresh from the farm (91.01%) (Schuphan, 1965) than in fresh spinach from markets (88.5-89.7%) (Holland et al., 1991; Martin-Villa et al., 1982). Generally the fresh spinach samples collected in this study had dark green color, crisp texture and appeared very acceptable in spite of the significant differences found in the concentrations of the free sugars, chlorophyll pigments and moisture.

REFERENCES

REFERENCES

Adsule, P.G. and Dan, A. 1979. Simplified Extraction Procedure in the Rapid Spectrophotometric Method for Lycopene estimation in Tomato. J. Food Science Technology 16:216.

Anonymous. 1989. Analysis of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection. Bulletin TN 20. Dionex Corporation Sunnyvale, CA., 23 pp.

AOAC. 1984. *Official Methods of Analysis.* 14th edition Association of Official Analytical Chemists. Washington D.C.

Bolin, H. and Huxsoll, C. 1991. Effect of permeation procedures and storage parameters on quality retention of salad cut lettuce. J. Food Sci., 56:60-62, 67.

Considine, D.M. (Ed.) and Considine, G.D. (Ed.), 1982. *Foods and Food Production Encyclopedia.* Van Nostrand Reinhold Company, New York, NY., p. 1887.

deMan, J.M. 1985. *Principles of Food Chemistry.* AVI Publishing Inc. Westport, Conn. 236-240.

Dutton, H.J., Baily, G.F.,and Kohake, E. 1943. Dehydrated spinach changes in color and pigments during processing and storage. Ind. Eng. Chem. 35, 1173-1177. Cited in Gross, J.(1991). *Pigments in Vegetables Chlorophylls and Carotenoids,* p. 36. Van Nostrand Reinhold Company, New York, NY.

Gross, J. 1991. *Pigments in Vegetables Chlorophylls and Carotenoids.* Van Nostrand Reinhold Company, New York, NY.

Holland, B., Unwin, I.D., Welch, A.A. and Buss, D.H. 1991. *Vegetables, Herbs and Spices: Fifth Supplement to McChance and Widdonson's The Composition of Foods.* HMSO, London, UK.

Iverson, J. and Bueno, M. 1981. Evaluation of high pressure liquid chromatography and gas-liquid chromatography for quantitative determination of sugars in foods. J. Assoc. Off. Anal. Chem.,64: 139-143.

Kaur, B., and Manjrekar, S.P. 1975. Effect of dehydration on the stability of chlorophyll and ß-carotine content of leafy green vegetables available in northern India. J. Food Sci. Technol. (India) 12, 321-323. Cited in Gross, J. (1991). *Pigments in Vegetables Chlorophylls and Carotenoids*, p. 36. Van Nostrand Reinhold Company, New York, NY.

Lapedes, D. (Editor), 1977. *McGraw-Hill Encyclopedia of Food, Agriculture and Nutrition.* McGraw Hill Book Company, New York, NY. Pg 625.

Lichtenthaler, H.K. 1987. Chlorophylls and carotinoids: pigments of photosynthetic biomembranes, in *Methods of Enzymology, Vol. 148, Plant Cell Membranes,* Ed. L. Packer and R. Douce, Academic Press, New York, NY.

Lotus Development Corporation 1985. *Lotus 1-2-3 (tm) Reference Manual, Release 2.01.* Cambridge, MA.

Martin-Villa, L. Videl-Velverde, C., and Hidalgo, R., 1982. HPLC determination of carbohydrates in raw and cooked vegetables. J. Food Sci. 47: 2086-2088.

Melton, S. 1993. Personal Communication. The University of Tennessee, Knoxville.

Mencarelli, F., Saltveit, M.E. Jr., 1988. Ripening of Mature-green Tomato Fruit Slices. J. Amer. Soc. Hort. Sci. 113(5):742-745.

Penfield, M. P. and Campbell, A.M, 1991. *Experimental Food Science.* (3rd. Edition). Academic Press, Inc., New York, New York.

Pomeranz, Y. and Meloan, C.E., 1987. *Food Analysis: Theory and Practice*. (2nd Edition). Van Nostrand Reinhold, New York.

Sanders, W. L. 1994. Personal Communication. The University of Tennessee, Knoxville.

SAS Institute Inc., 1982. *SAS User's Guide: Basics, 1982 Edition*. SAS Institute Inc., Cary NC, 923 pp.

Schuphan, W. 1965. *Nutritional Values in Crops and Plants.* Faber and Faber, London, UK.

Schwartz, S.J., and von Elbe, J.H., 1983. Kinetics of chlorophyll degradation to pyropheophytin in vegetables. J. Food Sci. 48. 1303-1306. Cited in Gross, J. (1991). *Pigments in Vegetables Chlorophylls and Carotenoids,* p. 23. Van Nostrand Reinhold Company, New York, NY.

Schwartz, S.J., Woo, S.L. and von Elbe, J.H. 1981. HPLC of chlorophylls and their derivatives in fresh and processed spinach. J. Agric. Food Chem. 29: 533-535.

Scott, F. 1992. HPLC determination of carbohydrates in foods. Chpt. 8 in *Food Analysis by HPLC.*(Ed.) Leo M.L. Nollet, 259-274. Marcel Decker Inc. New York.

Shaw, P.E. 1988. *Handbook of Sugar Seperations in Foods by HPLC*. CRC Press. Boca Raton, Fla. 20-42.

STSC Inc., 1991. Statgraphics (tm). *Reference Manual: Version 5.* Statistical Graphics Corporation. Rockville, Maryland.

Taylor, J.K. 1990. *Techniques For Data Analysis.* Lewis Publishing, Chelsea MI. pg 91.

Vernon, L.P. 1960. Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal. Chem. 32: 1144-1150.

Wills, R.B.H., McGlasson W.B., Graham, D., Lee, T.H., and Hall, G.E. 1989. *Postharvest. An Introduction to the Physiology and Handling of Fruit and Vegetables.* Van Nostrand Reinhold, New York, New York.

Yamauchi, N., Iida, S., Minamide, T., and Iwada, T., 1985. Foliage yellowing by peroxides in stored spinach (mechanism of chlorophyll degradation in harvested leaf vegetables, Part II). J. Jpn. Soc. Food Sci. Technol. 32, 814-817. Cited in Gross, J. (1991). *Pigments in Vegetables Chlorophylls and Carotenoids*. Van Nostrand Reinhold Company, New York, NY.

Zhu, S., Mount, J., and Collins J. 1992. Sugar and soluble solids changes in refrigerated sweet corn (Zea mays L). J. Food Sci. 57: 454-457.

APPENDICES

APPENDIX A

DIONEX METHOD PARAMETERS - CARBO.MET

Method Comment:
Column ID:
Analyst ID:

System Parameters

System Name: DX-300

Number of Detectors	1
Run Time (minutes)	20.0
Sampling Rate (seconds)	0.20
Detector 1 Type	PAD
Detector 1 real time plot scale maximum (nA)	1200
minimum	0.0
Detector 1 Output Equivalent to 1 Volt (in nA)	1000
Detector 1 ACI Analog Input Connection	DET1
Save Data File	Yes

Data File Name: C:\DX\DATA\acrodisk.D01

-- DETECTOR 1 PARAMETERS --

Report Options

Create ASCII Report File	No
Print Report	Yes
Print All Components	Yes
Print Components Found	No
Print Missing Components	No
Print All Peaks	No
Print Unknown Peaks	Yes
Print Chromatogram	Yes
Autoscale Chromatogram Maximum	No
Autoscale Chromatogram Minimum	No
Fill Peaks with Color	No
Draw Grid Lines on Chromatogram	No
Show Component Fraction Numbers	No
Label with Peak Number	Yes
Label with Retention Times on Chromatogram	No
Label with Component Name	Yes

Format File Name: C:\DX\METHOD\default.prf

Integration Parameters

Starting Peak Width (seconds)	20.0
Peak Threshold	5.00
Peak Area Reject	9999
Area Reject for Reference Peaks	1000

Data Events

Time	Description
0.00	Stop negative peak detection

Timed Events File: C:\DX\METHOD\CARBO.TE

Step	Time	Description
1	0.0	GPM Run Gradient Clock
1	0.0	GPM Reset OFF
2	0.1	ACI Autosmp OFF
2	0.1	PAD AutoOffset OFF

Piston Size: 1/8 inch
Lo Pressure Limit = 0
Hi Pressure Limit = 3000
Eluent 1 - HPLC Water
Eluent 2 - WATER
Eluent 3 - 200 mM NaOH
Eluent 4 - 200 mM NaOH
V5 Off - Load
V5 On - Inject
V6 Off - Off
V6 On - On

Time	Flow	%1	%2	%3	%4	Curve	V5	V6	Comment
0.0	1.00	0	20	80	0	5	0	1	
1.0	1.00	0	20	80	0	5	1	1	
20.0	1.00	0	20	80	0	5	0	1	

Component # 1 Glucose Retention Time 5.23
Reference Comp. none Window Size 5.00 %
Amount = K0 + K1*Area
K0 = 0.00000E+000
K1 = 1.42875E-006

Level	Amount	Area	Height
1	1.00000E+000	742162	81890
2	2.50000E+000	1791854	207017
3	5.00000E+000	3555108	405850
4	1.00000E+001	7041736	812025
5	1.50000E+001	10440772	1123857

Component # 2 Fructose Retention Time 5.73
Reference Comp. none Window Size 5.00 %
Amount = K0 + K1*Area
K0 = 0.00000E+000
K1 = 2.08728E-006

Level	Amount	Area	Height
1	1.00000E+000	538832	51637
2	2.50000E+000	1260305	109416
3	5.00000E+000	2478858	230402
4	1.00000E+001	4796098	433438
5	1.50000E+001	7138400	657478

Component # 3 Sucrose Retention Time 9.40
Reference Comp. none Window Size 5.00 %
Amount = K0 + K1*Area
K0 = 0.00000E+000
K1 = 3.25352E-006

Level	Amount	Area	Height
1	1.00000E+000	360123	19260
2	2.50000E+000	784829	41157
3	5.00000E+000	1579152	82653
4	1.00000E+001	3054686	161666
5	1.50000E+001	4601482	240183

Timed Events File: C:\DX\METHOD\CARBO.TE

Step	Time	Description
Init		ACI Autosmp OFF
Init		ACI RLY 2 OFF
Init		ACI TTL 1 OFF
Init		ACI TTL 2 OFF
Init		ACI AC 1 OFF
Init		PAD Cell ON
Init		PAD AutoOffset OFF
Init		PAD Recorder Mark OFF
Init		PAD Recorder Range = 1000.0 nA
Init		GPM Start
Init		GPM Hold Gradient Clock
Init		GPM Reset ON
1	0.0	ACI Autosmp ON
1	0.0	Start Sampling
1	0.0	PAD AutoOffset ON

Calibration Parameters

Number Of Levels for Calibration	5
Force Calibration Curve Through Origin	Yes
Calibration Fit Type	Linear
Replace Or Average Calibrations	Replace
External or Internal Calibration	External
Calculate Unknowns by Area or Height	Area
Default Sample Volume	1.0
Default Dilution Factor	1.0
Default Response Factor for Unknown Peaks	0.0
Calibration Standard Volume	1.0
Internal Standard Amount in Samples	1.0
Amount Units	

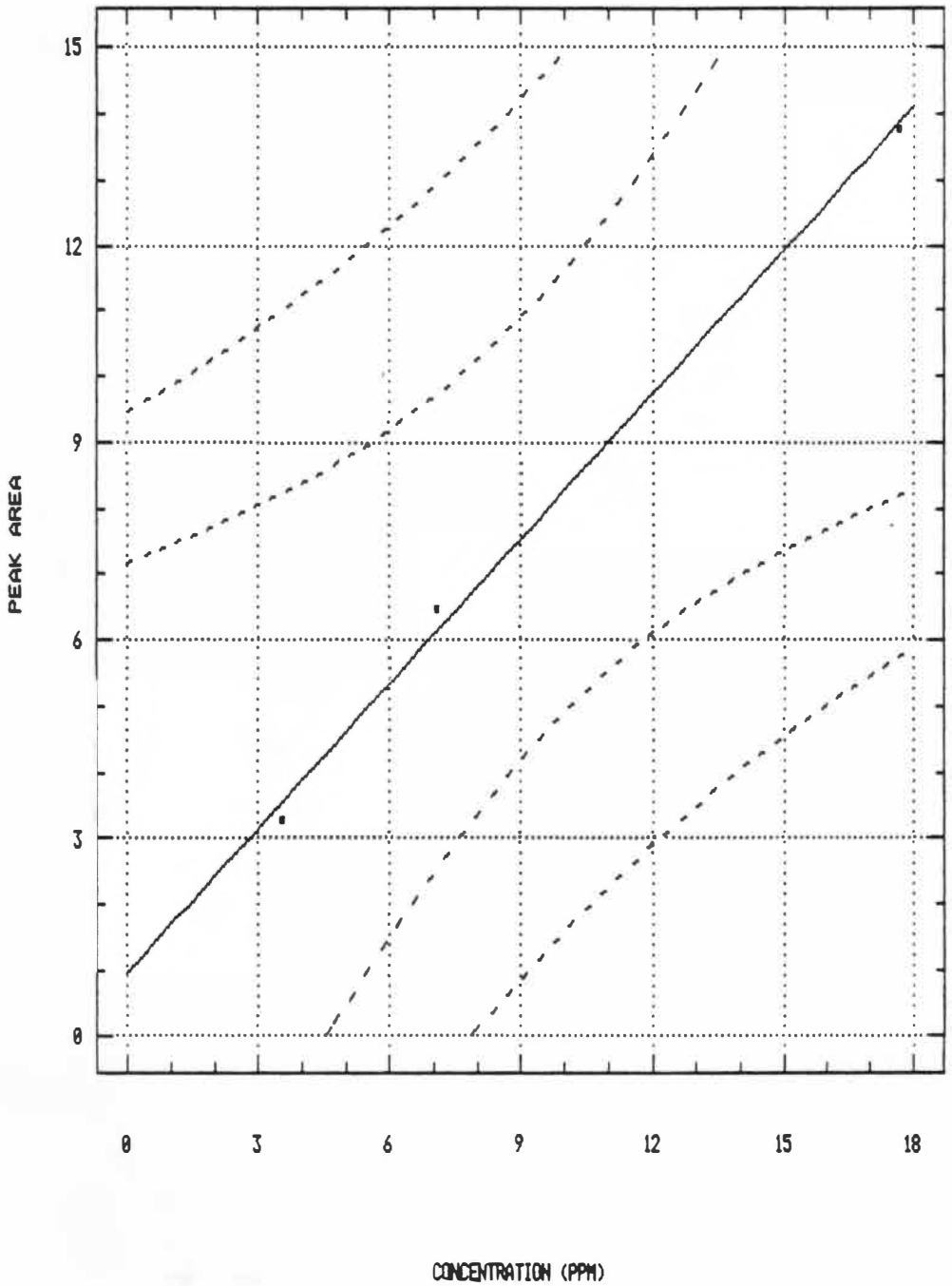


Figure A-1-Standard glucose curve, regression of peak area on concentration (PPM). (STSC Inc., 1991).

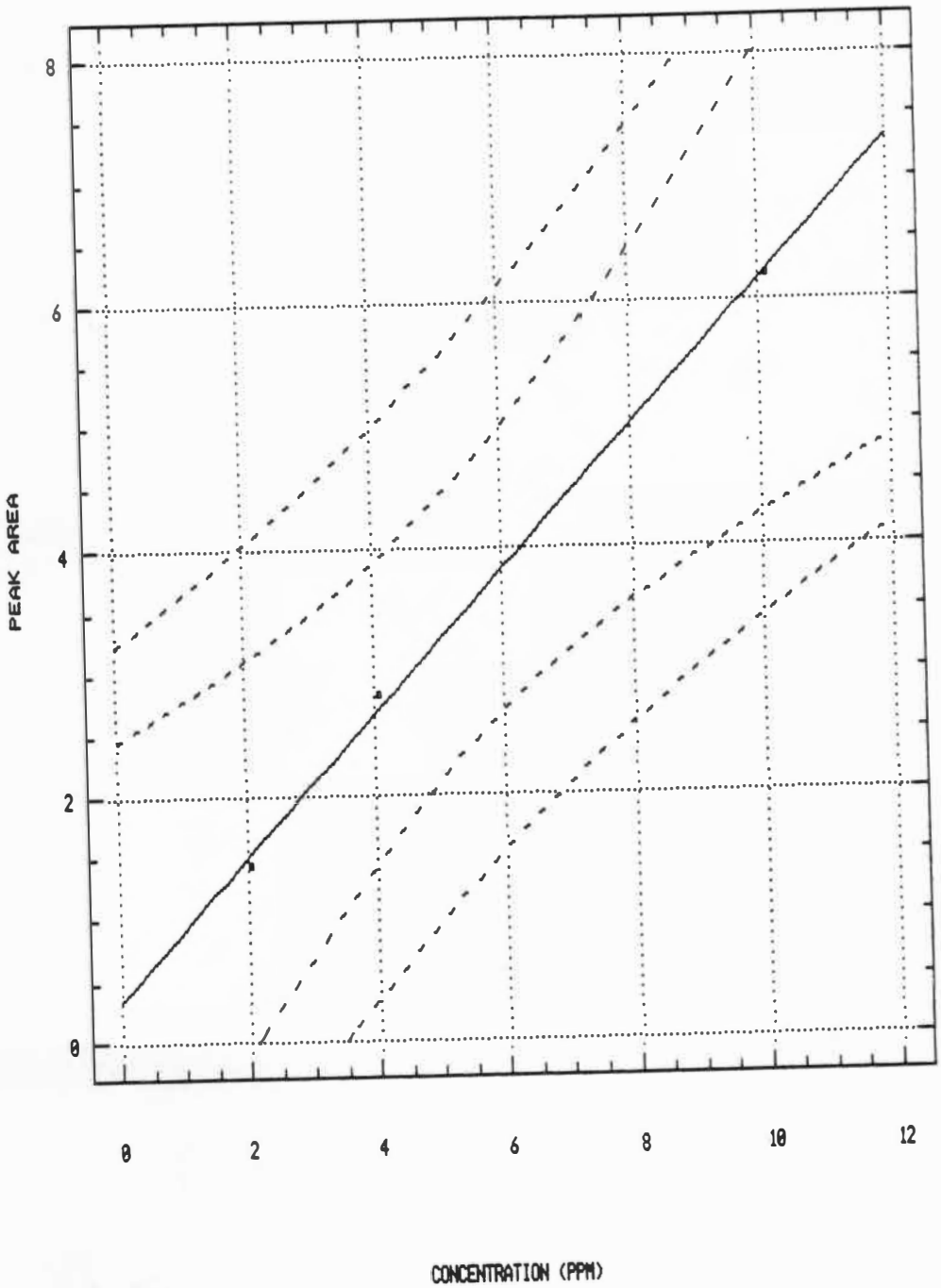


Figure A-2-Standard fructose curve, regression of peak area on concentration (PPM). (STSC Inc., 1991).

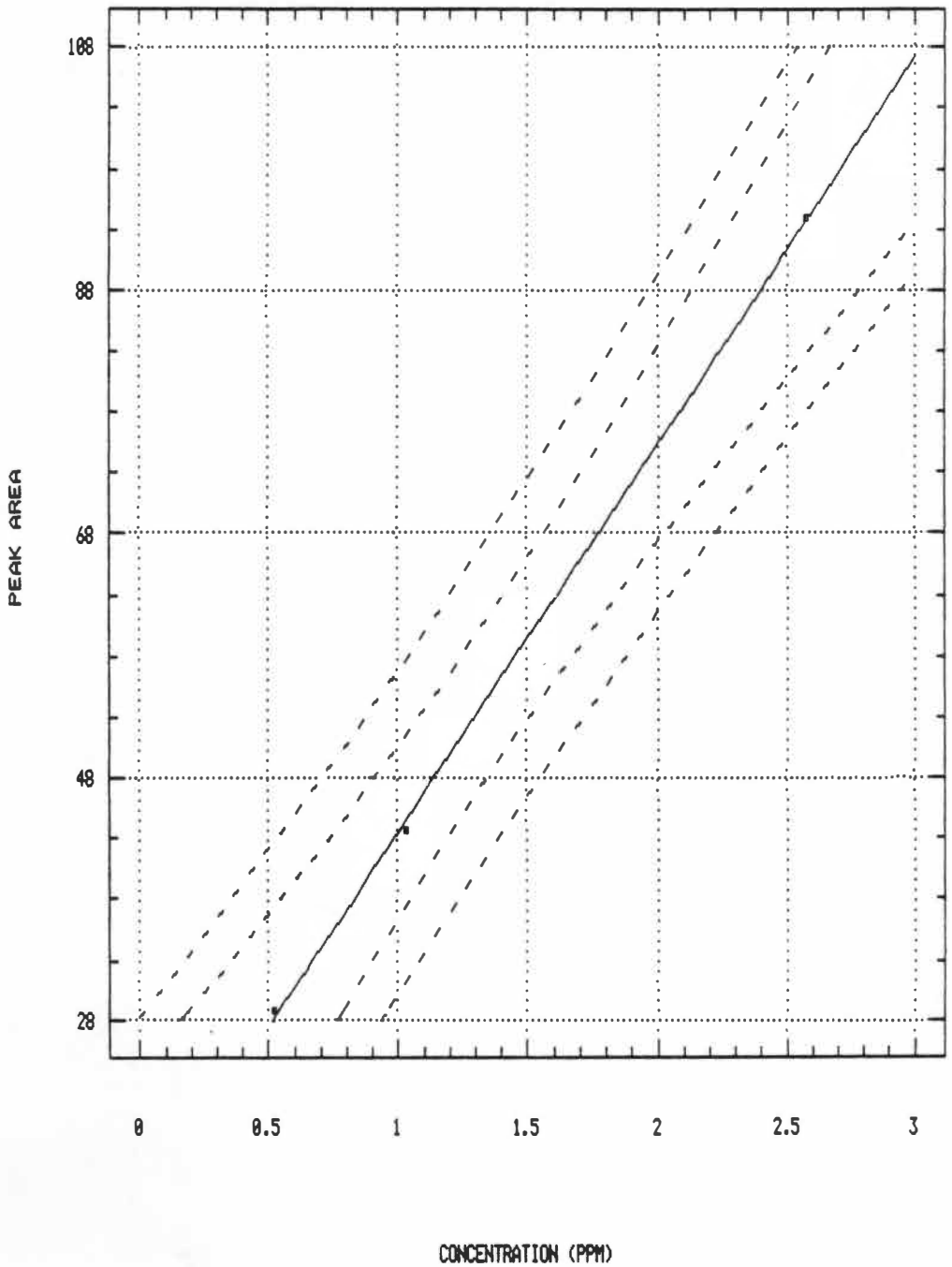


Figure A-3-Standard sucrose curve, regression of peak area on concentration (PPM). (STSC Inc., 1991).

Table A 1-Regression Analysis for Glucose Standard. Linear model: Y=a+bX

Dependent variable: Glucose Peak Area Independent variable: Concentration

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	970860	489217	1.98452	.29715
Slope	729837	43804.8	16.6611	.03816

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	5.7612E0013	1	5.7612E0013	2.776E0002	.03816
Residual	2.0754E0011	1	2.0754E0011		
Total (Corr.)	5.7820E0013	2			

Correlation Coefficient = 0.998204 R-squared = 99.64 percent

Stnd. Error of Est. = 455568

Table A 2-Regression Analysis for Fructose Standard. Linear Model: Y=a+bX

Dependent variable: Fructose Peak Area Independent variable: Concentration

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	352167	166195	2.11901	.28071
Slope	580800	25940.4	22.3898	.02841

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	1.1993E0013	1	1.1993E0013	5.013E0002	.02841
Residual	2.3923E0010	1	2.3923E0010		
Total (Corr.)	1.2017E0013	2			

Correlation Coefficient = 0.999004 R-squared = 99.80 percent

Stnd. Error of Est. = 154672

Table A 3-Regression Analysis for Sucrose Standard. Linear Model: Y=a+bX

Dependent variable: Sucrose Peak Area Independent variable: Concentration

Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	116809	9554.85	12.2251	.05196
Slope	318197	5855.63	54.3403	.01171

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	2.3308E0011	1	2.3308E0011	2.953E0003	.01171
Residual	78934239	1	78934239		
Total (Corr.)	2.3316E0011	2			

Correlation Coefficient = 0.999831 R-squared = 99.97 percent

Stnd. Error of Est. = 8884.49

Table A-4-Retention time results for each sugar in the standard mixture (including range, mean and standard deviation)

STANDARD SAMPLE	RETENTION TIME FOR EACH SUGAR IN STANDARD		
	GLUCOSE	FRUCTOSE	SUCROSE
A	5.3	5.83	9.43
B	5.3	5.83	9.47
C	5.3	5.83	9.5
RANGE	0.0	0.0	0.07
AVERAGE MEAN ($\bar{x}$)	5.3	5.83	9.47
SD	0.0	0.0	0.0287

Table A-5-Peak area and concentration data for standard sugar mixture

STANDARD SOLUTION	CONCENTRATION & PEAK AREA FOR EACH SUGAR IN MIXTURE					
	GLUCOSE		FRUCTOSE		SUCROSE	
	(ppm)	(Area)	(ppm)	(Area)	(ppm)	(Area)
A[a]	3.53	2714593	2.030	1297553	0.52	173459
B[b]	7.06	6404735	4.050	2938622	1.03	443153
C[c]	17.66	16058648	10.130	8455603	2.58	1111102

[a] Standard A was diluted, .1 mL (standard) brought to 200 mL with deionized water.
[b] Standard B was diluted, .1 mL (standard) brought to 100 mL with deionized water.
[c] Standard C was diluted, 1 mL (standard) brought to 400 mL with deionized water.

Table A-6-Chromatographic data from anion exchange HPLC analysis of sugars extracted from fresh spinach. Samples 4,5,6,7 and 8 were spiked prior to extraction procedure to determine % recovery

SPINACH SAMPLE	GLUCOSE PEAK AREA	PPM	FRUCTOSE PEAK AREA	PPM	SUCROSE PEAK AREA	PPM
1	2886747	2.625	2075680	2.9675	403045	0.8996
2	2166548	1.6383	1523249	2.0163	306480	0.5961
3	2224274	1.7174	1556796	2.0741	335076	0.6859
4 Spiked[a]	3375787	3.2952	2438430	3.592	813307	2.1889
5 Spiked[b]	6221861	7.1948	4573345	7.2679	1887675	5.5653
6 Spiked[c]	5269942	5.8905	3863244	6.0452	1677297	4.9042
7 Spiked[d]	6521636	7.6055	4841989	7.7304	2244942	6.6881
8 Spiked[e]	7759366	9.3014	5931818	9.6068	2804652	8.4471
9	2400132	1.9583	1681491	2.2888	349907	0.7326
10	2652767	2.3045	1919846	2.6992	397906	0.8834

[a] Sample #4, 20% spiking sugars/80% deionized water added.
[b] Sample #5, 60% spiking sugars/40% deionized water added.
[c] Sample #6, 40% spiking sugars/60% deionized water added.
[d] Sample #7, 80% spiking sugars/20% deionized water added.
[e] Sample #8, 100% spiking sugars (no dilution) added.

APPENDIX B

Table B-1-Analyses of variance for glucose contents in spinach from four locations[a] (markets) in samples collected for (a) eight weeks[b] during the spring of 1995 and (b) for weeks 1-4 and (c) weeks 5-8 during that same period

(a) Eight weeks sampling

General Linear Models Procedure

Dependent Variable: **Glucose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.0319358	0.0106453	0.28	0.8398
WK	7	0.6905845	0.0986549	2.59	0.0317
WK*LOC	21	0.8675562	0.0413122	1.08	0.4100
Error	31	1.1808060	0.0380905		
Corrected Total	62	2.7663483			

(b) Weeks 1 - 4 sampling

General Linear Models Procedure

Dependent Variable: **Glucose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.0869561	0.0289854	1.97	0.1586
WK	3	0.4595201	0.1531734	10.43	0.0005
WK*LOC	9	0.1352316	0.0150257	1.02	0.4627
Error	16	0.2349760	0.0146860		
Corrected Total	31	0.9166839			

(c) Weeks 5 - 8 sampling

General Linear Models Procedure

Dependent Variable: **Glucose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.0149653	0.0049884	0.08	0.9703
WK	3	0.0219189	0.0073063	0.12	0.9494
WK*LOC	9	0.6667331	0.0740815	1.17	0.3758
Error	15	0.9458300	0.0630553		
Corrected Total	30	1.6594097			

[a]Loc = locations (Bilo, Food Lion, Kroger, Sams).
[b]Wk = weeks of sampling.

Table B-2-Analyses of variance for fructose contents in spinach from four locations[a] (markets) in samples collected for (a) eight weeks[b] during the spring of 1995 and (b) for weeks 1-4 and (c) weeks 5-8 during that same period

(a) Eight weeks sampling

General Linear Models Procedure

Dependent Variable: **Fructose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.1203408	0.0401136	1.78	0.1708
WK	7	0.3723039	0.0531863	2.36	0.0466
WK*LOC	21	0.5365214	0.0255486	1.14	0.3659
Error	31	0.6972220	0.0224910		
Corrected Total	62	1.6630823			

(b) Weeks 1 - 4 sampling

General Linear Models Procedure

Dependent Variable: **Fructose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.0440084	0.0146695	0.44	0.7266
WK	3	0.2854311	0.0951437	2.86	0.0695
WK*LOC	9	0.1235394	0.0137266	0.41	0.9097
Error	16	0.5317730	0.0332358		
Corrected Total	31	0.9847519			

(c) Weeks 5 - 8 sampling

General Linear Models Procedure

Dependent Variable: **Fructose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.1887823	0.0629274	5.71	0.0082
WK	3	0.0865136	0.0288379	2.61	0.0894
WK*LOC	9	0.3153923	0.0350436	3.18	0.0234
Error	15	0.1654490	0.0110299		
Corrected Total	30	0.6778254			

[a]Loc = locations (Bilo, Food Lion, Kroger, Sams).
[b]Wk = weeks of sampling.

Table B-3-Analyses of variance for sucrose contents in spinach from four locations[a] (markets) in samples collected for (a) eight weeks[b] during the spring of 1995 and (b) for weeks 1-4 and (c) weeks 5-8 during that same period

(a) Eight weeks sampling

General Linear Models Procedure

Dependent Variable: **Sucrose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.1517551	0.0505850	3.28	0.0339
WK	7	0.3910802	0.0558686	3.62	0.0058
WK*LOC	21	0.6140339	0.0292397	1.90	0.0516
Error	31	0.4782245	0.0154266		
Corrected Total	62	1.6154333			

(b) Weeks 1 - 4 sampling

General Linear Models Procedure

Dependent Variable: **Sucrose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.2153265	0.0717755	4.41	0.0192
WK	3	0.2022250	0.0674083	4.15	0.0237
WK*LOC	9	0.2105870	0.0233986	1.44	0.2517
Error	16	0.2601890	0.0162618		
Corrected Total	31	0.8883275			

(c) Weeks 5 - 8 sampling

General Linear Models Procedure

Dependent Variable: **Sucrose**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.1157435	0.0385812	2.65	0.0863
WK	3	0.0056403	0.0018801	0.13	0.9412
WK*LOC	9	0.2240437	0.0248937	1.71	0.1715
Error	15	0.2180355	0.0145357		
Corrected Total	30	0.5543714			

[a]Loc = locations (Bilo, Food Lion, Kroger, Sams).
[b]Wk = weeks of sampling.

Table B-4-Analyses of variance for total sugar contents in spinach from four locations[a] (markets) in samples collected for (a) eight weeks[b] during the spring of 1995 and (b) for weeks 1-4 and (c) weeks 5-8 during that same period

(a) Eight weeks sampling

General Linear Models Procedure

Dependent Variable: **Total sugars**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.6641094	0.2213698	1.32	0.2849
WK	7	2.9716810	0.4245259	2.54	0.0348
WK*LOC	21	4.1802995	0.1990619	1.19	0.3241
Error	31	5.1908675	0.1674473		
Corrected Total	62	12.7432963			

(b) Weeks 1 - 4 sampling

General Linear Models Procedure

Dependent Variable: **Total sugars**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.8797352	0.2932451	1.80	0.1872
WK	3	2.0309237	0.6769746	4.16	0.0234
WK*LOC	9	0.8969060	0.0996562	0.61	0.7696
Error	16	2.6025330	0.1626583		
Corrected Total	31	6.4100980			

(c) Weeks 5 - 8 sampling

General Linear Models Procedure

Dependent Variable: **Total sugars**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.7679500	0.2559833	1.48	0.2592
WK	3	0.1234973	0.0411658	0.24	0.8681
WK*LOC	9	2.3578324	0.2619814	1.52	0.2280
Error	15	2.5883345	0.1725556		
Corrected Total	30	5.6460327			

[a]Loc = locations (Bilo, Food Lion, Kroger, Sams).
[b]Wk = weeks of sampling.

Table B-5-Analyses of variance for chlorophyll a content in spinach from four locations[a] (markets) in samples collected for (a) eight weeks[b] during the spring of 1995 and (b) for weeks 1-4 and (c) weeks 5-8 during that same period

(a) Eight weeks sampling

General Linear Models Procedure

Dependent Variable: **Chlorophyll a**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.1604339	0.0534780	3.43	0.0293
WK	7	0.8496602	0.1213800	7.80	0.0001
WK*LOC	21	0.8333453	0.0396831	2.55	0.0094
Error	30	0.2523970	0.0084132		
Corrected Total	61	1.3461639			

(b) Weeks 1 - 4 sampling

General Linear Models Procedure

Dependent Variable: **Chlorophyll a**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.1164057	0.0388019	6.56	0.0054
WK	3	0.2718183	0.0906061	15.33	0.0001
WK*LOC	9	0.2321841	0.0257982	4.36	0.0071
Error	14	0.0827615	0.0059115		
Corrected Total	29	0.7340907			

(c) Weeks 5 - 8 sampling

General Linear Models Procedure

Dependent Variable: **Chlorophyll a**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.0823241	0.0274414	2.59	0.0890
WK	3	0.1363966	0.0454655	4.29	0.0212
WK*LOC	9	0.1518693	0.0168744	1.59	0.2000
Error	16	0.1696355	0.0106022		
Corrected Total	31	0.5402255			

[a]Loc = locations (Bilo, Food Lion, Kroger, Sams).
[b]Wk = weeks of sampling.

Table B-6-Analyses of variance for chlorophyll b contents in spinach from four locations[a] (markets) in samples collected for (a) eight weeks[b] during the spring of 1995 and (b) for weeks 1-4 and (c) weeks 5-8 during that same period

(a) Eight weeks sampling

General Linear Models Procedure

Dependent Variable: **Chlorophyll b**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.0138305	0.0046102	4.03	0.0160
WK	7	0.0683926	0.0097704	8.54	0.0001
WK*LOC	21	0.0510720	0.0024320	2.13	0.0287
Error	30	0.0343080	0.0011436		
Corrected Total	61	0.1762275			

(b) Weeks 1 - 4 sampling

General Linear Models Procedure

Dependent Variable: **Chlorophyll b**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.0158231	0.0052744	5.89	0.0081
WK	3	0.0331627	0.0110542	12.35	0.0003
WK*LOC	9	0.0206739	0.0022971	2.57	0.0554
Error	14	0.0125345	0.0008953		
Corrected Total	29	0.0870310			

(c) Weeks 5 - 8 sampling

General Linear Models Procedure

Dependent Variable: **Chlorophyll b**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.0132541	0.0044180	3.25	0.0497
WK	3	0.0236918	0.0078973	5.80	0.0070
WK*LOC	9	0.0167710	0.0018634	1.37	0.2793
Error	16	0.0217735	0.0013608		
Corrected Total	31	0.0754905			

[a]Loc = locations (Bilo, Food Lion, Kroger, Sams).
[b]Wk = weeks of sampling.

Table B-7-Analyses of variance for total chlorophyll content in spinach from four locations[a] (markets) in samples collected for (a) eight weeks[b] during the spring of 1995 and (b) for weeks 1-4 and (c) weeks 5-8 during that same period

(a) Eight weeks sampling

General Linear Models Procedure

Dependent Variable: **Total chlorophyll**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.1604339	0.0534780	3.43	0.0293
WK	7	0.8496602	0.1213800	7.80	0.0001
WK*LOC	21	0.8333453	0.0396831	2.55	0.0094
Error	30	0.4671165	0.0155706		
Corrected Total	61	2.4242869			

(b) Weeks 1 - 4 sampling

General Linear Models Procedure

Dependent Variable: **Total chlorophyll**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.2151560	0.0717187	6.40	0.0059
WK	3	0.4671325	0.1557108	13.89	0.0002
WK*LOC	9	0.3770440	0.0418938	3.74	0.0137
Error	14	0.1569720	0.0112123		
Corrected Total	29	1.2772619			

(c) Weeks 5 - 8 sampling

General Linear Models Procedure

Dependent Variable: **Total chlorophyll**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	0.1610663	0.0536888	2.77	0.0755
WK	3	0.2627141	0.0875714	4.52	0.0177
WK*LOC	9	0.2646900	0.0294100	1.52	0.2237
Error	16	0.3101445	0.0193840		
Corrected Total	31	0.9986150			

[a]Loc = locations (Bilo, Food Lion, Kroger, Sams).
[b]Wk = Weeks of sampling.

Table B-8-Analyses of variance for moisture contents in spinach from four locations[a] (markets) in samples collected for (a) eight weeks[b] during the spring of 1995 and (b) for weeks 1-4 and (c) weeks 5-8 during that same period

(a) Eight weeks sampling

General Linear Models Procedure

Dependent Variable: **Moisture**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	8.930556	2.976852	11.06	0.0001
WK	7	17.296894	2.470985	9.18	0.0001
WK*LOC	21	13.534244	0.644488	2.39	0.0127
Error	32	8.615000	0.269219		
Corrected Total	63	48.376694			

(b) Weeks 1 - 4 sampling

General Linear Models Procedure

Dependent Variable: **Moisture**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	8.1632344	2.7210781	56.33	0.0001
WK	3	0.9927344	0.3309115	6.85	0.0035
WK*LOC	9	4.2465781	0.4718420	9.77	0.0001
Error	16	0.772850	0.048303		
Corrected Total	31	14.175397			

(c) Weeks 5 - 8 sampling

General Linear Models Procedure

Dependent Variable: **Moisture**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
LOC	3	3.757434	1.252478	2.56	0.0917
WK	3	12.203534	4.067845	8.30	0.0015
WK*LOC	9	6.297553	0.699728	1.43	0.2560
Error	16	7.842150	0.490134		
Corrected Total	31	30.100672			

[a]Loc = locations (Bilo, Food Lion, Kroger, Sams).
[b]Wk = weeks of sampling.

Table B-9-Spinach survey correlation analysis.
(SAS Institute, 1982)

Variable	Moisture	Glucose	Fructose	Sucrose	Total Sugar	Total Chlor.	Chl. a	Chl. b
Moisture	1.00	-.15	-.35*	-.32*	-.31*	.44*	.42*	.45*
Glucose		1.00	.53*	.65*	.89*	-.22	-.23	-.19
Fructose			1.00	.51*	.78*	-.07	-.06	-.09
Sucrose				1.00	.85*	-.44*	-.42*	-.47*
Total Sugar					1.00	-.29*	-.28*	-.23*
Total Chlor.						1.00	.99*	.96*
Chl. a							1.00	.93*
Chl. b								1.00

*p<.05
N=61

Table B-10-General statistics. (SAS Institute, 1982).

Simple Statistics

Variable	N	Mean	Std Dev	Sum
CHAB	62	0.871403	0.199355	54.027000
CHA	62	0.644742	0.148554	39.974000
CHB	62	0.226677	0.053749	14.054000
GLU	63	0.265651	0.211231	16.736000
FRU	63	0.126651	0.163780	7.979000
SUC	63	0.289413	0.161417	18.233000
TS	63	0.681794	0.453362	42.953000
MOI	64	91.152187	0.876290	5833.740000

Simple Statistics

Variable	Minimum	Maximum
CHAB	0.386000	1.378000
CHA	0.269000	1.026000
CHB	0.117000	0.375000
GLU	0.005000	1.167000
FRU	0	0.712000
SUC	0	0.787000
TS	0.005000	1.712000
MOI	88.940000	92.630000

VITA

Harvey J. Drews was born in Brooklyn, New York on April 22, 1941. He attended the public schools at the elementary and high school level in the Borough of Queens, New York. In 1958, he entered The State University of New York at Farmingdale and in 1960 he received an Associate Degree in Applied Science in Frozen Food Technology. In September of 1960 he entered The University of Georgia at Athens and in May of 1963, received a Bachelor of Science in Agriculture with a major in Food Science and Technology. He is planning to receive the Master of Science Degree in Agriculture with a major in Food Science and Technology in May of 1996. His past work experience includes:

1991-1994 Technical Sales Food Broker, L.L. Brown & Company. 1987-1990 Director of Operations, Phyton Technologies. 1985-1987 Consultant, Food Technics Inc. 1977 -1985 Director of R & D, White Lily Foods. 1977-1978 Scientist, Shakey's Pizza Inc. 1976-1977 Consultant, Dynamore Corp. 1973-1976 Director of Manufacturing, Western Baker's Supply Co. 1969-1973 Tech. Service Mgr., Great Western United Co. 1966-1969 Food Technologist, Kraft Foods Inc. 1965-1966 Chemist, American Chicle. 1963-1965 Chemist, Sunshine Biscuit Company.

His professional affiliation includes the Institute of Food Technologists. His professional accomplishments include being a holder of US patent # 3,671,264 for the invention of " Formula's and process for making a fruit flavored nugget." and of US patent # 3,592,360 for the invention of " Formulation and method for making complete dry dressing mixes."