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A NEW METHOD FOR DETERMINING
FRUCTOSE AND GLUCOSE IN POTATOES

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NEW METHOD FOR DETERMINING FRUCTOSE AND GLUCOSE IN POTATOES

By

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ABSTRACT

A NEW METHOD FOR DETERMINING FRUCTOSE AND GLUCOSE IN POTATOES

By

Ahmet Fatih Tarhan

Certain problems are often encountered during the application of established methods of quantitative determination of fructose and glucose in potato samples. Furthermore, such methods are extremely time consuming. This has led to development of a new method to accurately determine the concentration of fructose and glucose, whereby tedious sample preparations could be omitted.

The methodology involves the use of an "Anthrone Colorimetric Method" to obtain standard curves for fructose, glucose and different combinations of both, to derive an empirical formula from those standard curves. This procedure utilizes glucose oxidase to eliminate glucose in the sample, after which the concentration of fructose is determined from a standard curve and the concentration of glucose is calculated from an empirical formula.

This method has been successfully applied to six different varieties of potatoes as well as apple samples. The results were in very good agreement with the results of the "Official Lane-Eynon Method" when they were compared.

To Gönül and Taner

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INTRODUCTION

The commonly occuring crystallizable sugars in food materials belong to the classes of mono and disaccharides. Some of the monosaccharides, which are carbohydrates that cannot be hydrolyzed to simpler molecules, form the basic monomers from which carbohydrate polymers such as starch, pectin and cellulose are derived.

Monosaccharides are classified according to the length of the carbon chain and whether there is an aldehyde or ketone group present. If the molecule contains an aldehyde group, it is termed an aldose, while one with a ketone group is termed a ketose.

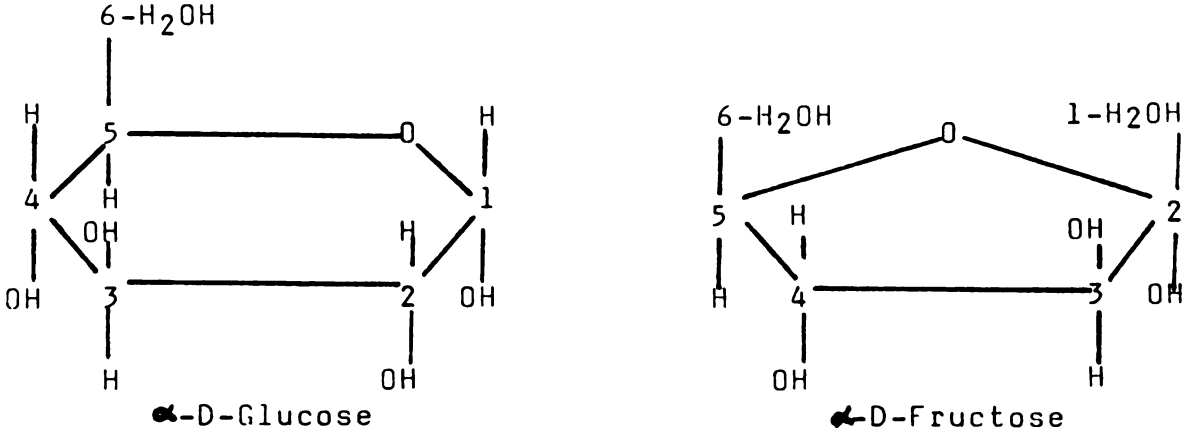

Figure 1. Structural formulas for Glucose and Fructose. Numbers refer to the carbon atoms.

The "plane" of the Haworth ring is a conventionalized presentation, since the actual molecules are three-dimensional. The attachments at the top of the vertical lines are considered to be above the plane of the ring, those at the bottom, below the ring. The 6-membered pyranose or 5-membered furanose ring can open by breaking between the oxygen and C_5 to give reducing-sugar properties responsible for certain nonenzymatic browning reactions through amine: sugar condensation (e.g., the browning of potato chips).

Glucose occurs free in nature in many plant materials and is also widely distributed in animals as the principal transport form of carbohydrate in the blood system. One of the early names for glucose was "grape sugar," because it was found in fairly large quantities in grapes. It also occurs in combined form in sucrose, lactose, maltose and polysaccharides such as starch, cellulose and glycogen.

Free fructose is found primarily in plants and materials derived from plants such as honey. Fructose is one of the two monomers in sucrose, and the basic monomer of the polysaccharide inulin. Fructose is the sweetest of the sugars, as well as the most water soluble.

Sugars influence many properties of foods in addition to flavor. They alter the degree of hydration of many substances, influence the viscosity of starch pastes,

the firmness of gelatin and pectin gels, and the formation and strength of gluten strands. Sugars also play a role in browning reactions which occur widely in foodstuffs. The colors produced in this type of reacion range from pale yellow to dark brown or black depending on the type of product and the extent of reaction. In many foods the colors produced are considered desirable, for example, the brown crust of baked products and the color of caramel, maple syrup or peanut brittle. In other foods browning is detrimental, as in the darkening of dehydrated fruits, vegetables, eggs, and canned or dried milk. Browning reactions may be either enzymatic or nonenzymatic. Many of the enzymatic types are recognized in fruits and vegetables and involve the oxidation of polyphenolic compounds mediated by oxidative enzymes in plant cells, but the nonenzymatic browning reactions are the ones which frequently involve sugars or sugar related compounds.

Maillard (48) was the first to describe the development of a brown color in mixtures containing amino acids and reducing sugars. The brown materials produced during the reaction appear to be mostly melanoidins although it is likely that other by products may make slight contributions to the brown color. Later it was shown that proteins as well as amino acids may react with the

reducing sugars (64) to produce the browning reaction. Hodge (37) and Reynolds (65) list a wide variety of compounds that may participate in these reactions as presented in Figure 2.

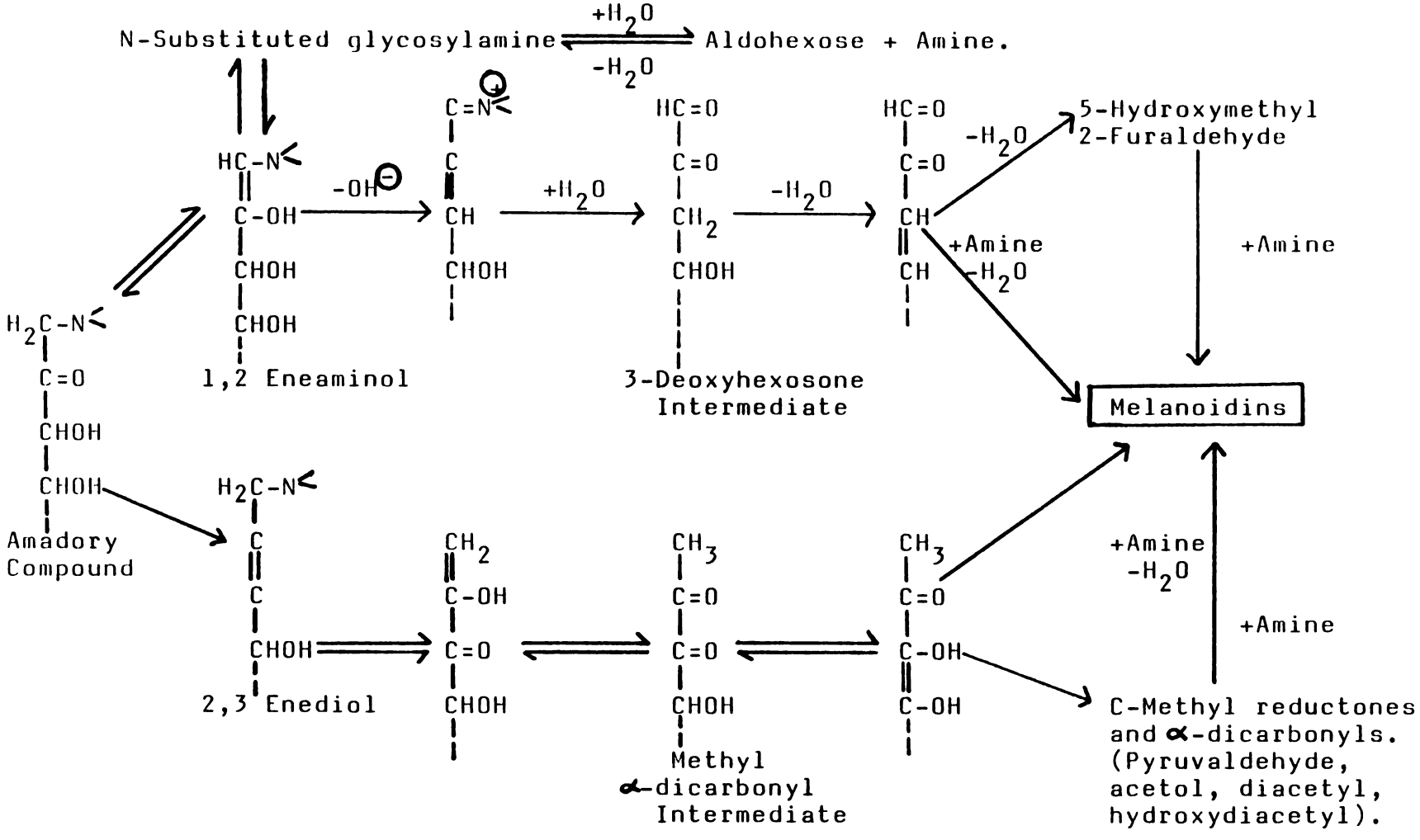

Figure 2. Sugar-amine (Maillard) browning reactions: two pathyways to melanoidins and by products (24).

LITERATURE REVIEW

Methods Used in Separation and Analysis of Sugars

For the qualitative determination of the individual sugars, many different tests have been proposed, based upon the reactivity of carbonyl groups, oxidative splitting of vicinal glycol groups, and on color reactions of the strong acid degradation products with organic compounds. Browne and Zerban (13) classified the sugars into reducing sugars and nonreducing sugars on the basis of their reaction with Fehling's solution (Alkaline cupric tartrate). The reducing sugars, which possess a free anomeric center, reduce the cupric ion to a red cuprous oxide precipitate. The nonreducing sugars, of which sucrose the common example are without action on Fehling's solution. Classical analytical methods utilize the reducing properties of sugars in the quatitative determination of these compounds. Newer methods are based on color reactions of some reagent with the sugar itself or with a furfurol type of degradation product of sugars in strong acid. Physicochemical methods such as polarography, polarimetry and others have also been used. Recently, specific

enzyme methods have become available. A Glucostat test kit containing a buffer, reduced chromogen, catalase and glucose oxidase is commercially available (Worthington Biochemical Corporation, Freehold, N.J.) for the quantitative colorimetric analysis of glucose. The mutarotated α, β-D-glucose sample is incubated with the Glucostat solution and the absorbance of the color measured spectrophotometrically.

Chromatographic methods are used to separate and analyze mixtures of sugars in biological materials. The development of chromatography and its applications in sugar separations have placed unique demands on methods for determining the reducing sugars. The reliability of the analysis for a particular sugar depends mostly on the efficiency with which the sugar is separated from a mixture of other sugars. (40)

Paper Chromatography:

With this technique a mixture of sugars is separated on a paper sheet by irrigation with an organic solvent mixture. The sheet is dried, and the areas occupied by the various sugars are revealed by dipping or spraying the paper sheet with a substance that reacts with sugars to produce a color. The intensities of the colored spots are read directly by a densitometer and compared with standards of known sugars run simultaneously.

Paper chromatography was introduced by Partridge and Westall (58, 59). Bevenue and Williams (6) used an indicator dip of 4, 5-dinitroveratole for direct ultramicrodetermination of sugars on paper chromatograms. Coleman et al. (18) determined glucose quantitatively in egg solids by paper chromatography much more accurately than could be determined by the copper reduction method. The introduction of paper chromatography is important for the study of oligosaccharides because it permits separations of complex mixtures. Paper chromatography for oligosaccharides is similar to that for monosaccharides except development time is often longer but excellent resolution can be obtained. Details about paper chromatography are available from Bell (5), Cassidy (16), Block et al. (10), Heftmann (35), Hough and Jones (38), Bailey and Pridham (3) and Stanek et al. (73).

Thin-Layer Chromatography:

Thin-layer chromatography which was introduced by Kirchner et al. (44) in 1951 is a technique similar to paper chromatography except the support is silica gel or some absorbent spread on a glass plate. The major advantage of this method is its speed (development is usually complete in an hour or less), but it has the disadvantage of being able to handle only small amounts of sample. Several different materials may be used as adsorbent, but

silica gel is used far more often than any other. Cellulose is also frequently used and Wolfrom et al. (83) reported that cellulose materials gave more effective separation of water-soluble sugars and sugar derivatives than silica gel. They also indicated that the same solvent systems used for paper chromatography could be satisfactorily used for cellulose thin-layer chromatography.

In 1970 Nagasawa et al. (57), using thin-layer chromatography with cellulose as adsorbent and aqueous perchloric acid as the solvent, described a systematic investigation of the color reaction of sugars, sugar derivatives, and related compounds. They also explored the relationship between the chemical structure of sugars and the sensitivity of the color reaction, and the application of this reaction to the detection of sugars on cellulose thin-layer.

Column Chromatography:

This technique consists of loading a sample solution at the top of a tube containing a chromatographic support and developing with an appropriate solvent. The separated sample is then excised from the solid column or collected in fractions as the separated compounds are eluted from the column. Jones et al. (39) have indicated that using cation exchange resins as the chromatographic material and water as the eluant provides one of the most effective column techniques for sugar separations. A good review of column

chromatography of carbohydrates is given by Binkley and Wolfrom (7).

Gas-Liquid Partition Chromatography:

Gas chromatography of sugars as their volatile methyl esters was first reported by McInnes et al. (53). A gas chromatograph consists of a thermostatically controlled oven, columns packed with various inert supports and liquid phases to serve as the chromatographic agent, a detector system, amplifier, and strip chart recorder. The most exacting problem in a gas chromatograph is presented by the sample injection system. The sample must be introduced as a vapor in the smallest possible volume and in a minimum amount of time without either decomposing or fractionating the sample or upsetting the equilibrium conditions of the column. Methyl glycosides of monosaccharides fulfilled these requirements and were the first group of carbohydrate derivatives investigated. Oligosaccharide esters can also be separated by using programmed temperatures. With gas chromatography techniques, fractions can be analyzed quantitatively in a few minutes. Gas chromatographic techniques have also been used for quantitative determinations of monosaccharides during fermentation (50), in fruits (45, 17), in fruit juices (74), in wheat and wheat products (51), and in enzyme inverted corn syrups (79). Detailed reviews on the separations of sugar

derivatives by gas chromatography are given by Kircher (43) and Bishop (8, 9).

Chemical Methods For Determining Monosaccharides:

Among the many methods available and applicable for sugar analysis only a few are used for food analysis. The Munson Walker copper reduction method (56) is used for macrodeterminations, but the Somogyi copper micro-method (71) is most widely used for general research work. The general procedure for either of these methods consists of oxidizing the sample by heating carefully with a freshly mixed solution of alkaline cupric tartrate. Cuprous oxide precipitates as the sugar is oxidized and the oxide is determined by any of several methods such as, gravimetrically, by electrolytic deposition, by titration with sodium thiosulfate or by reaction with ferric ions and titration with potassium permanganate. The basic equation for this reaction is:

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

$$Cu_2O + 2H^+ + I_2 \longrightarrow 2Cu^{2+} + 2I^- + H_2O$$

$$I_2 + 2S_2O_3^{2-} \longrightarrow 2I^- + S_4O_6^{2-}$$

A copper reduction method is described for the determination of sugar in silages and forages by Wiseman et al. (82). Hefferan and Goodnight (34) determined glucose and glycogen from a sample of meat using a modified Somogyi copper method.

Colorimetric Methods:

Colorimetric methods have become popular in recent years because they are simple and are applicable to very small amounts of materials. In 1926 Campbell and Hanna (15) published a method for determination of fructose in blood and urine based upon the reduction of molybdenum in phosphoric acid solution and the reoxidation of the reduced molybdenum with potassium permanganate. In 1946 Dreywood (22) introduced the anthrone-sulfuric acid color test for carbohydrates that is in wide use today, both as a qualitative and quantitative method. In 1968 the anthrone method was modified by Handel (33) for use in determining sucrose in potatoes. In 1956 Dubois et al. (23) developed the phenol-sulfuric method for sugar determination which was simple, rapid, sensitive, accurate and specific for carbohydrates. Both the anthrone and phenol-sulfuric methods require a separate standardization curve for each sugar being measured. In 1965 Rohwer et al. (66) developed the Glucostat method for determining glucose. This method was dilute solutions of sugar hydrolysates which are reacted with a duo-enzyme preparation of "Glucostat Special" consisting of a buffer, reduced chromogen, catalase and glucose oxidase. This method was adapted to the determination of glucose in starch hydrolysates, corn syrups, and sugar solutions. In 1967 Garret and Blanch (30) developed a sensitive direct spectrophotometric method for fructose

and sucrose determination after acid degradation. In 1967 Braun and Wadman (12) determined microamounts of iodine and glucose with fluorescein, while Potter et al. (61) used an atomic absorption spectrophotometer for quantitative analysis of reducing sugars by determining unreduced copper. In 1974 Monica et al. (54) measured the fructose content of fruits and potatoes as the difference between total reducing sugars and the glucose concentration. This method used alkaline ferricyanide and glucose oxidase for measuring total reducing sugars and glucose. This investigation determined sucrose by measuring the increase in glucose content after acid hydrolysis of the disaccharide. In 1974 McCready et al. (52) measured sugar, starch and amylose in potatoes by using an automated analysis method which employs a sugar-dinitrosalicylate and amylose-iodine reaction. In 1975 Vandercook et al. (78) determined total sugars, total acidity, total amino acids and phenolics in orange juice by using an automated method similar to McCready's. Quantitative colorimetric methods have been summarized and reviewed by Bell (5), Dische (20, 21), Hodge and Hofreiter (36), Stanek et al. (73), and Montreuil and Spik (55).

Zone Electrophoresis:

Zone electrophoresis also known as inophoresis, inography, electromigration, or electrochromatography has been

used for separating electrically charged materials using a support, buffers and electric current. The manipulations are in many ways similar to paper chromatography except for the actual development of the chromatograms in the migration chamber. The advantages of this procedure over paper chromatography lies in its ability to separate sugars that migrate together on paper with the usual solvents. For example D-Galactose, D-Mannose and D-Fructose are not always readily separable by paper chromatography in the usual solvents but are easily separated by zone electrophoresis. The use of very high potential gradients has further improved separations by this method (31). A good review of zone electrophoresis of carbohydrates has been presented by Foster (25, 26), Block et al. (10), Zweig (84) and Wieland (81).

Specific Enzyme Methods:

Specific chemical qualitative tests to differentiate between all of the individual monosaccharides important in foods are not available. For free glucose in foods a good qualitative and quantitative test has been devised using glucose oxidase, β-D glucose: O_2 oxireductase which oxidizes β-D-glucopyranose (27, 42). Although glucose oxidase (GOD) oxidizes α-D-glucose much more slowly than β-D-glucose (42), the enzyme can still be used to determine total D-glucose because α-glucose is converted spontaneously to β-glucose as the latter is removed from the

system. Glucose was first determined with GOD manometrically (41) and then later colorimetrically (28, 76). The optimum conditions, limits of error and range of application for the routine determination of glucose in blood have been extensively studied by Schon and Bucke (69) and Raabo at al. (63). Since disaccharides are not present in blood the "true glucose" value is obtained even with impure glucose oxidase preparations, but for the analysis of mixtures of sugars, it is necessary to use highly purified enzyme preparations. Glucose in a number of materials, including blood, urine (49), corn syrup (80), hydrolysates of polysaccharides (68), and fermentation liqours (19) has been determined with glucose oxidase. The principle of the technique is summarized below:

$$\text{D-Glucose} \xrightarrow[\text{oxidase}]{\text{glucose}} \text{D-Gluconic acid lactone} + \text{Hydrogen peroxide}$$

$$\text{Hydrogen peroxide} \xrightarrow{\text{catalase}} \text{Water} + \text{Oxygen}$$

$$\text{Oxygen} + \text{reduced leuko chromogen} \longrightarrow \text{oxidized colored chromogen (blue)}$$

Bostic and Hercules (11) determined glucose in blood by using a chemiluminescent enzyme method. An automated analysis system consisting of a flow-thru electrode assembly, glucose oxidase and molybdate catalyst has been described for the determination of glucose in protein loaded serum samples (47). In this technique molybdate is substitute for the

expensive peroxide enzyme used in the coupled glucose oxidase-peroxidase enzyme system and as a result the need for color development has been eliminated. Another automated analysis method was developed by Gaines (29) who determined glucose and fructose in potatoes by using glucose oxidase and invertase. Avigad et al. (2) determined D-galactose by using a coupled enzyme system containing galactose oxidase. The principle of the method is shown below:

$$\text{D-Galactose} + O_2 \xrightarrow[\text{oxidase}]{\text{galactose}} \text{D-Galacto-hexodialdose} + \text{hydrogen peroxide}$$

$$\text{Hydrogen peroxide} \xrightarrow{\text{peroxidase}} \text{Oxygen} + \text{Water}$$

$$\text{Oxygen} + \text{Leuko chromogen (colorless)} \longrightarrow \text{Oxidized chromogen (colored)}$$

Galactose oxidase and also Galactostat, a test kit for quantitative analysis of galactose and galactose containing sugars are commercially available.

Sugars of Potato Tuber

The sugar content of potatoes (solanum tuberosum) may vary from only trace amounts to as much as ten percent of the dry weight of the tuber and thus 1/3 to 1/2 of the non-starch solids (4). Freshly harvested mature tubers may contain only traces of sugar, whereas certain varieties of tubers harvested prior to full maturity may have as much as 1.5 percent sugar and small tubers usually contain higher percentages

of sugar than do large tubers (46). Variety and temperature are the main factors which influence sugar content of potatoes during post-harvest storage with varieties having a low specific gravity, generally accumulating more sugar than varieties of high specific gravity (75). At storage temperatures below about 50 F, the total and reducing sugar increase with the rate and extent of increase becoming greater as the temperature falls toward the freezing point (75). According to older literature (75), sucrose, glucose and fructose are usually present in the potato in approximately equal amounts however, more recent work (75) indicates that during the initial stages of storage at low temperatures, sucrose seems to accumulate most rapidly and upon prolonged storage the ratio of sucrose to reducing sugar tends to increase with decreasing temperature (14). The dominating reducing sugar in cold stored immature potato is fructose (67). When tubers which have been stored at low temperatures are conditioned at higher temperatures, the sugar content gradually decreases over a period of 3 to 4 weeks. Since the percentage of sugar decreases and percentage of starch increase during conditioning, it has been tacitly assumed that the sugar is reconverted into starch (75). Pressey (62) found that freshly harvested potatoes contain low levels of the enzyme invertase, which hydrolyzes sucrose to simple sugars, as well as high levels of invertase inhibitor. Total invertase was found to increase sharply

when potatoes were placed in cold storage but the inhibitor was not depleted. Results indicated that invertase participated in reducing sugar formation, but other factors were responsible for the starch-sugar conversion in potatoes during storage at low temperatures (75). It is assumed that the reducing power of potatoes prepared for analysis is solely attributed to glucose and fructose, and that these, together with sucrose make up the total sugars, however, trace amounts of other sugars have been detected in potatoes. These include maltose, xylose, sugar phosphates, raffinose, melibiose, heptulose and melezitose (32). Several non-sugar components such as tyrosine, ascorbic acid, cysteine, gluthathione and inositol have also been found in potatoes (70). In 1978 Toma et al. (77) reported the ranges of the proximate composition and caloric values of potatoes. (Table 1).

Table 1. Proximate Composition and Caloric Values of Potatoes (77)

Components	Range (g/150g Fresh Weight)
Moisture	110.84-123.96
Total Ash	1.04 1.88
Crude Fiber	0.50 0.94
Total Carbohydrates	21.0 35.60
Protein	2.48 4.09
Caloric Value	94.0 158.90

Potatoes high in sugar taste sweet and have a poor texture when cooked. In the manufacture of potato chips, French fries and dehydrated potatoes, the sugar content is closely related to the color produced during the processing procedure, and in the case of dehydrated products, to the darkening which may take place during subsequent storage. The source of the yellow to brown color of these products is attributed to the Maillard or non-enzymatic browning reaction between the aldehyde groups of reducing sugars and the free amino groups of the amino acids, and, perhaps to a lesser degree of the proteins of the potato. This would indicate that the controlling factor in determining the amount of browning is the reducing sugar rather than the total sugar content. As a rule, potatoes containing more than two percent reducing sugars on a dry weight basis are considered to be unacceptable for most kinds of processing (75). In order to secure suitable raw material of low browning tendency, it is the general practice to use cultivars which are poor sugar formers and to process potatoes in storage at periods during which they are at low sugar level and have not sprouted. This may be accomplished by conditioning cold storage tubers for 2 to 3 weeks at room temperature (18^{o}C), or by storing potatoes at 10^{o}C (75).

The purpose of this study was to develop a simple and

efficient method for determining individual reducing sugar content and to utilize this method for the analysis of several potato cultivars in an effort to predict storability and process ability of these potatoes.

MATERIALS AND METHODS

Preparation of Standard Curves:

An anthrone colorimetric method (36) was used to obtain standard curves for glucose, fructose and various combinations of glucose and fructose.

Standard glucose-fructose solutions were prepared by dissolving 100 mg glucose or fructose in 100 ml of distilled, deionized (DI) water. Appropriate dilutions of the glucose and fructose standards were prepared and then combined as follows:

80 mg Glucose + 20 mg Fructose = 100 mg mixture/100 ml.

60 mg Glucose + 40 mg Fructose = 100 mg mixture/100 ml.

50 mg Glucose + 50 mg Fructose = 100 mg mixture/100 ml.

40 mg Glucose + 60 mg Fructose = 100 mg mixture/100 ml.

20 mg Glucose + 80 mg Fructose = 100 mg mixture/100 ml.

One ml of each of the solutions of glucose, fructose or combinations of these sugars, plus four ml of distilled DI water were placed in optically matched tubes which were then immersed in $10^{o}C$ water. Ten ml of anthrone solution, containing 200 mg anthrone/100 ml of concentrated H_2SO_4, were added to each tube, which was then shaken on a vortex

mixer to thoroughly mix the contents. The blank consisted of five ml of distilled DI water plus 10 ml of anthrone solution. To obtain maximum color development, the tubes were heated for 16 minutes in boiling water then cooled to room temperature in cold water. Absorbance values were determined, using a spectrophotometer (Baush and Lomb Spectronic 70) set at 625 nm. All absorbance readings were made within one hour of color development because anthrone solutions tend to be unstable over prolonged periods of time. Standard curves for glucose, fructose and glucose-fructose combinations were obtained by plotting absorbances versus concentrations (μg/ml) of each individual reducing sugar (i.e., glucose and fructose) and combinations of total reducing sugars. (Figure 1 and 2). Slope values for each standard curve were calculated and natural logarithms of the inverse slope values were taken and plotted against fractions of glucose and fructose. This plot resulted in a straight line. (Figure 3). It can be shown (Fig. 2) that:

$$\text{Absorbance} = \text{Slope} \times \text{Concentration}$$

$$\boxed{A = m \times C} \qquad (1)$$

Where A = Absorbance for unknown mixture

m = Slope

C = Concentration of Total Reducing Sugar (μg/ml)

And:

$$\ell n\frac{1}{m} = \text{y intercept} + \text{Slope} \times \text{Glucose fraction}$$

$$\boxed{\ell n\frac{1}{m} = 4.7 + 0.7 \times X_G} \qquad (2)$$
(Figure 3)

where X_G = Glucose Fraction

From equation (1), $m = \frac{A}{C}$ which can be inserted into equation (2) to give:

$$\boxed{\ell n\frac{C}{A} = 4.7 + 0.7 \times X_G} \qquad (3)$$

Since, $\boxed{C = C_G + C_F}$ (4)

C_G = Concentration of glucose.

C_F = Concentration of fructose.

C = Total Concentration.

Equation (3) can be rearranged as follows:

$$\boxed{\ell n\left(\frac{C_G + C_F}{A}\right) = 4.7 + 0.7 \times X_G} \qquad (5)$$

Since, $C_G = X_G \times C$ and $C_F = X_F \times C$ and $C = C_G + C_F$

$$\boxed{X_G = \frac{C_G}{C} = \frac{C_G}{C_G + C_F}} \qquad (6)$$

When X_G is inserted into equation (5)

$$\boxed{\ell n\left(\frac{C_G + C_F}{A}\right) = 4.7 + 0.7\left(\frac{C_G}{C_G + C_F}\right)} \qquad (7)$$

or,

$$2.303 \log \left(\frac{C_G + C_F}{A} \right) = 4.7 + 0.7 \left(\frac{C_G}{C_G + C_F} \right)$$

represents the empirical equation sought.

Recovery and Comparison With Other Techniques:

Recovery experiments were done using solutions containing 60 μg glucose and 40 μg fructose/ml. For the enzyme assay, 25 ml of solution were mixed with 25 ml of buffer which contained 350 units of glucose oxidase enzyme. A second recovery experiment was conducted with the new technique. Potatoes were macerated in an Acme juicerator and 300 ml of juice were collected. This juice was equally divided and transferred into 3 different beakers. To one beaker 100 mg of glucose was added, to a second beaker 100 mg of fructose, and to a third beaker 50 mg of glucose + 50 mg of fructose were added and mixed until sugars were completely dissolved. Each sample was analyzed separately for glucose and fructose and then percentage recoveries were determined. (Table 3).

The technique described herein was compared with the official Lane Eynon copper reduction method in potato and

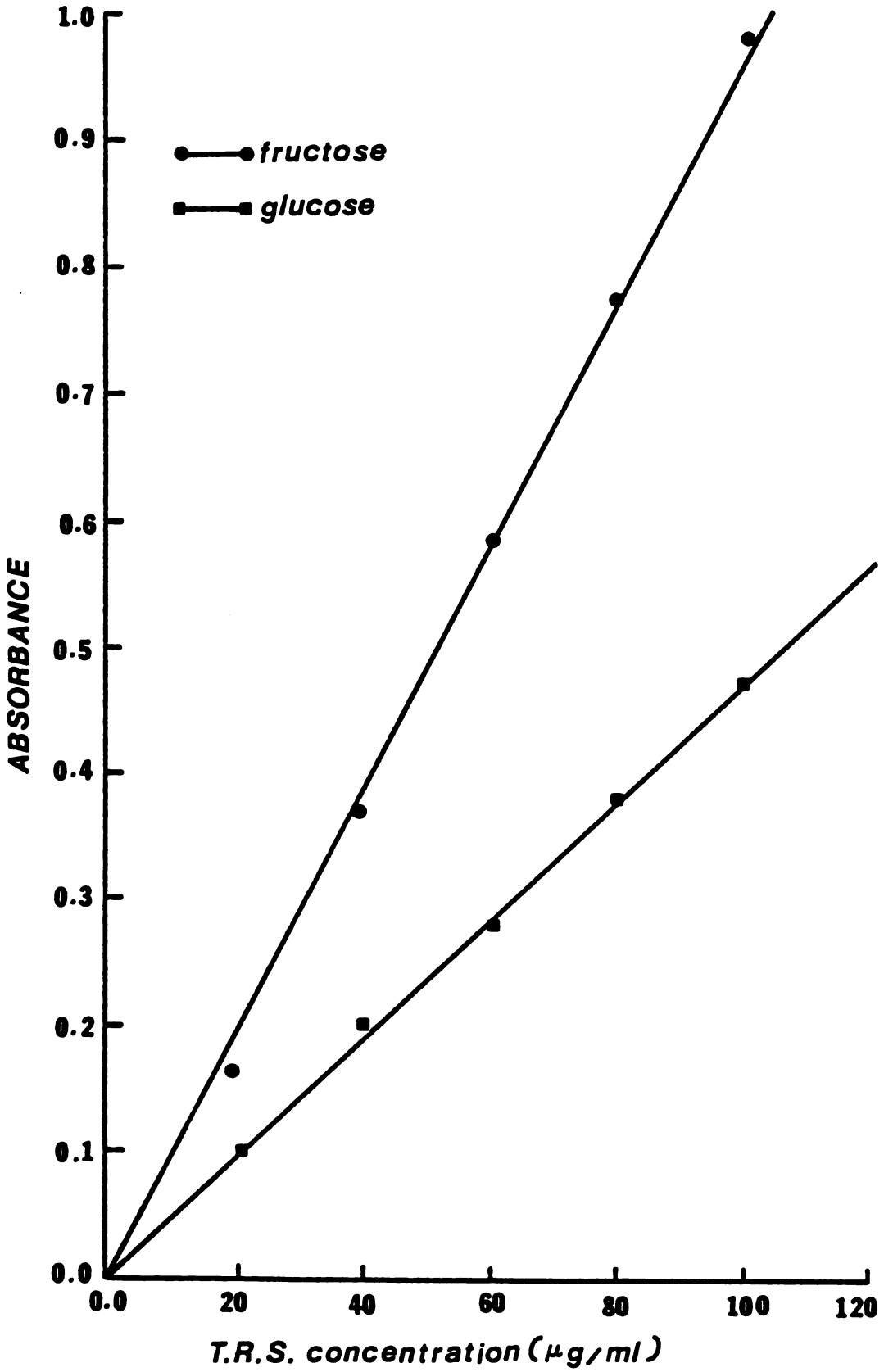

Figure 3. Standard curves for glucose and fructose.

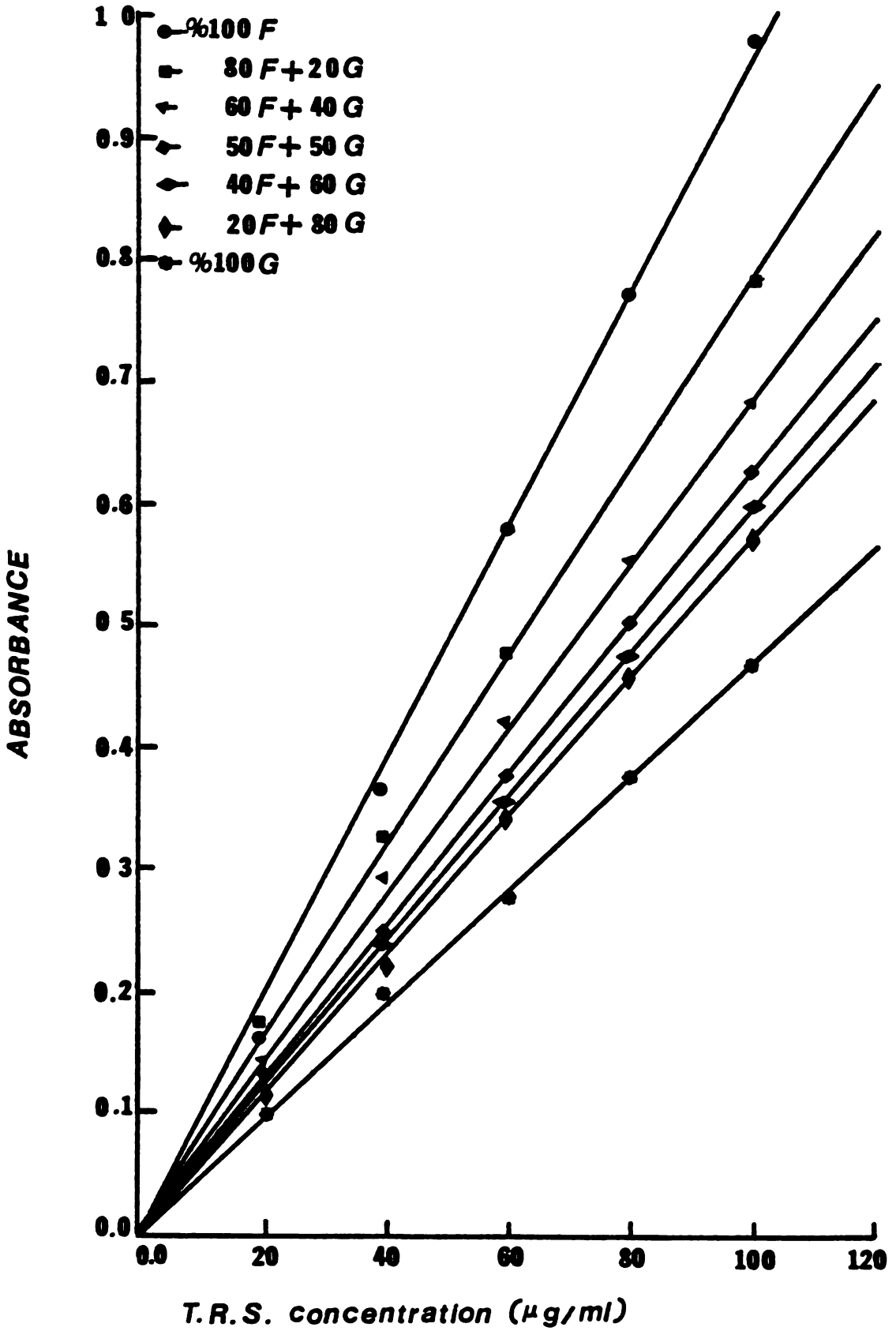

Figure 4. Standard curves for glucose, fructose and different glucose-fructose combinations.

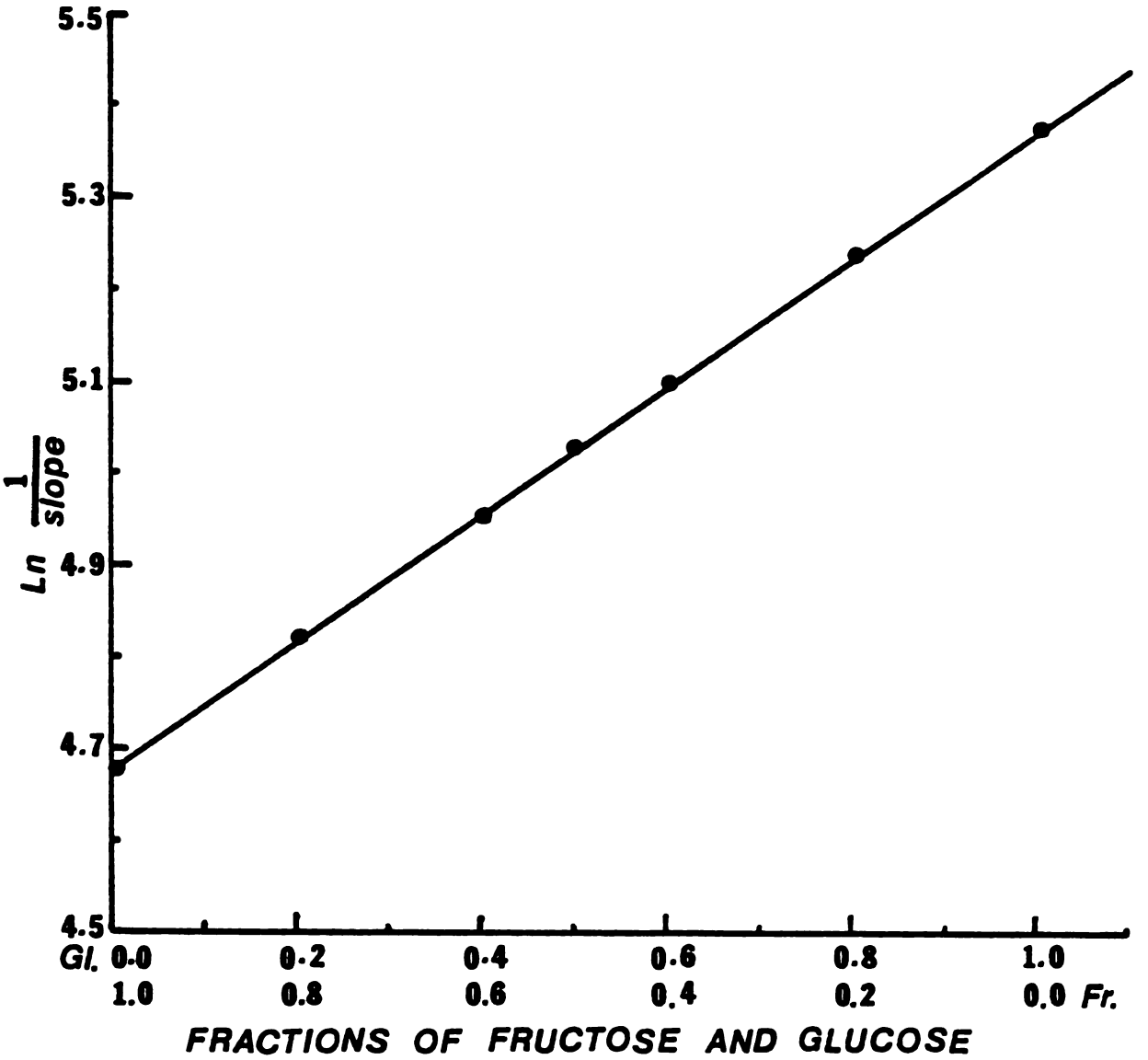

Figure 5. Plot of natural logarithms of inverse slope values versus fractions of glucose and fructose.

apple samples. Samples were prepared for analysis according to the Lane Eynon procedure and analyzed by Lane Eynon and the new method. Total reducing sugars were determined for the potato and apple samples and the results from both methods were compared. (Table 4.)

Analysis of Potatoes with the New Technique:

The potato samples analyzed came from Norchip, Belchip, Atlantic, Superior, Denali and Michibonne varieties which were harvested at two different times. The first harvest was August 9th, 1978 and the second harvest was October 20th, 1978. For each variety a 200 g sample was collected by taking longitudinal center slices from 6-8 different tubers. This 200 g of potato tissue was macerated for juice in an Acme juicerator and the juicerator was washed with 300 ml of distilled, DI water which was added to the juice. The juice was immediately frozen and held at -26^{o} C until analysis could be performed.

Before analysis the sample was thawed and diluted to 430 ml with distilled, DI water. Five ml of sample were further diluted to 50 ml for analysis. Five ml of diluted sample were mixed with 1.5 ml of enzyme solution which had been prepared by dissolving 10 mg of glucose oxidase (P.L. Biochemicals, Inc.) in 10 ml KH_2PO_4 buffer, pH 6.0. (0.5 M KH_2PO_4 - 0.5 M NaOH, [10:1 v/v]) to give a solution which contained 100 units of glucose oxidase activity per

milliliter. This enzyme-sample solution was allowed to react at ambient room temperature for 30 minutes to destroy the glucose present in the juice sample. The reaction time necessary for the enzyme to destroy the glucose in the juice sample had been previously determined by assessing the initial concentration of glucose--fructose in an appropriately diluted sample, using the anthrone method as previously described for the preparation of standard curves. The value obtained at this point is designated as "Total Absorbance (A)." Five ml of diluted sample were then mixed with 1.5 ml of enzyme solution which contained 100 units/ml of activity. This mixture was stirred vigorously and 1.0 ml samples were withdrawn at 10 minute intervals for color development with the anthrone solution. When the color of the samples no longer changed (i.e., absorbance at 625 nm became steady) the oxidation of glucose in the sample was complete and the time necessary for this to happen was chosen as the reaction time. If a shorter reaction time was desired, more enzyme was used.

After completion of the oxidation of glucose, the absorbance value obtained with the anthrone method was used with the fructose standard curve to find the fructose concentration (C_F) of the dilute sample. When the fructose concentration and total absorbance values are inserted into

the empirical equation, the glucose concentration (C_G) of the dilute sample can be determined by trial and error substitution of possible glucose values into the equation until a value is found which allows the equation to equal zero. For mg/ml in fresh potato juice, the dilution factor would be:

$$\frac{5 \times 10}{1000} = \underline{\underline{0.05}}$$

For mg/g fresh potato, the dilution factor would be:

$$\frac{10 \times 430}{200 \quad 1000} = \underline{\underline{0.0215}}$$

As a result:

Glucose concentration (C_G) = C_G x 0.05 mg glucose/ml fresh juice.

or = C_G x 0.0215 mg glucose/g fresh potato.

Fructose concentration (C_F) = C_F x 0.05 mg fructose/ml fresh juice.

or = C_F x 0.0215 mg fructose/g fresh potato.

Total Reducing Sugar Concentration (C_T) = $C_G + C_F$

RESULTS AND DISCUSSION

Most colorimetric techniques for reducing sugars utilize a standard curve for glucose or fructose although the total reducing sugar composition may be a combination of these sugars. This is not necessarily a problem if the study is comperative in nature, however, if it is necessary to quantitate each component sugar, a standard curve for glucose is not adequate. As can be seen in figures 1 and 2 the anthrone absorbance values for equal concentrations of fructose and glucose are different. Only when the ratio of these two sugars in a mixture is known it is possible to convert an anthrone absorbance value to total reducing sugar. One of the important aspects of current research with potatoes is the determination of the individual reducing sugar changes at harvest and during storage; therefore it becomes necessary to devise a method for measuring these changes.

Table 2 shows the absorbance value before enzymatic reaction and the changes in absorbance values during the enzymatic reaction of the recovery experiment.

Since the original mixture of 60 μg glucose--40 μg

Table 2. Absorbance values at 10 minute intervals during recovery experiment.

Time (Minute)	ABSORBANCES at 625 nm.		
	Replication I	Replication II	Average
0	.600	.600	.600
10	.258	.250	.254
20	.210	.238	.224
30	.200	.200	.200
40	.200	.200	.200
50	.200	.200	.200

fructose was dominated by glucose a considerable reduction in absorbance value after oxidation of glucose was anticipated. (Figure 1). Using the standard curve for fructose it was found that the absorbance value of 0.200 corresponded to 20 μg/ml of fructose. (Figure 1). Considering the dilution factor of 2 (25 ml of sample solution + 25 ml of buffer-enzyme solution), this would give 40 μg/ml of fructose in the mixture, so it is obvious that the enzyme has oxidized all the glucose. Since this is total recovery of fructose, it can be assumed that glucose oxidase did not have any effect on the fructose in the solution. When this fructose concentration (40 μg/ml) and the absorbance value before assay (0.600) are inserted into equation (7), the glucose concentration is found to be 60.5 μg/ml. The 0.5 μg/ml difference can be attributed to experimental error. Although

it might be expected that the anthrone absorbance values for glucose and fructose should be additive when the sugars are mixed, recovery experiments showed that, the anthrone absorbance values for fructose and glucose are not additive. However, the empirical equation was able to provide total recovery of glucose.

Results from recovery experiments with a potato sample indicate a recovery range of 96.3-102.9% for fructose and a recovery range of 98.7-99.7% for glucose. (Table 3).

Table 3. Recovery of glucose and fructose added to a potato sample. Glucose and fructose concentrations are given as μg/ml of potato juice.

	Expected Concentration		Glucose Determined	% Glucose Recovery	Fructose Determined	% Fructose Recovery
	Glucose	Fructose				
Addition of 1000 μg/ml Glucose	1170	600	1166	99.7	586	97.7
Addition of 1000 μg/ml Fructose	170	1600	168	98.8	1540	96.3
Addition of 500 μg/ml Glucose + 500 μg/ml Fructose	670	1100	661	98.7	1132	102.9

Some of the results from the analysis of potatoes and also some apple samples with the new technique and the Lane-Eynon official method are shown in the table 4. For potato samples both methods are in close agreement.

Table 4. Results (mg/g fresh weight) from analysis of potato and apple samples with the new and official techniques.

	THE NEW TECHNIQUE			Lane-Eynon	
Product	Glucose	Fructose	Total Reducing Sugar	Total Reducing Sugar	% Difference
Potato	0.416	1.470	1.886	1.875	+0.0012
Apple	19.940	70.490	90.430	92.0	-0.157

However, for the apple, there is a 1.57 mg/g difference which may be due to experimental errors. The Lane-Eynon official method gives total reducing sugars as dextrose but it does not detect glucose or fructose individually and it cannot detect less than milligram amounts. The new method described herein can differentiate between glucose and fructose in microgram quantities. These comparisons indicate that the new technique may have several advantages over the copper reduction techniques which have traditionally been viewed as official methods.

Table 5 shows the results from analysis of six different varieties of potatoes with the new technique. Potatoes from the early harvest are at less than optimum maturity and it might be expected that the fructose levels would

be high. This proved to be the case in the Norchip, Belchip, Michibonne, Denali and Superior varieties but in Atlantic the fructose-glucose levels were about equal. The glucose level in Denali was as high as that of Atlantic but its fructose level was much higher.

The potatoes harvested in October were more mature and analysis of these samples indicates that there is decreases in the amount of fructose for all varieties as compared to their fructose content at the immature stage. The rate of the decrease was higher in Superior, Denali and Atlantic than other varieties. There was also a drastic decrease in the amount of glucose for Norchip, Belchip, Superior, Atlantic and Denali, but a considerable increase in glucose in the Michibonne variety.

Table 5. Fructose, Glucose and Total Reducing Sugar Contents (mg/g fresh weight) of Several Potato Varieties at 2 harvest dates.

	HARVEST DATE: August 9, 1978			HARVEST DATE: October 20, 1978		
POTATO VARIETY	GLUCOSE	FRUCTOSE	TOTAL REDUCING SUGAR	GLUCOSE	FRUCTOSE	TOTAL REDUCING SUGAR
NORCHIP	0.301	1.064	1.365	<0.002	0.925	0.925
BELCHIP	0.372	1.312	1.684	0.00234	0.834	0.836
MICHIBONNE	0.546	1.150	1.696	2.612	0.914	3.526
SUPERIOR	0.828	1.892	2.720	0.045	0.667	0.712
ATLANTIC	1.489	1.419	2.908	<0.002	0.645	0.645
DENALI	1.489	2.258	3.747	0.282	1.258	1.540

SUMMARY

A colorimetric method was developed which allows the determination of glucose and fructose individually in natural or artificial mixture of the two sugars. The color is formed by the reaction of anthrone with the two sugars. First the total anthrone absorbance is measured, then the glucose of the mixture is destroyed by adding glucose oxidase, and the anthrone absorbance of the remaining fructose is determined. By means of a reference curve the anthrone absorbance of fructose is converted to fructose concentration and by means of a formula the glucose concentration of the mixture is calculated.

The new procedure was applied to the determination of glucose and fructose present in several potato cultivars and one apple cultivar. The values for total reducing sugars obtained by the new procedure were in excellent agreement with the values obtained by the Lane-Eynon method.

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APPENDICES

APPENDIX A

COMPUTER PROGRAM FOR CALCULATING OF GLUCOSE USING THE EMPIRICAL EQUATION. (BASIC COMPUTER LANGUAGE).

APPENDIX A

COMPUTER PROGRAM FOR CALCULATING OF GLUCOSE USING THE EMPIRICAL EQUATION (BASIC COMPUTER LANGUAGE)

$$C_G - Ae^{\left(4.7 + 0.7 \frac{C_G}{C_G + C_F}\right)} + C_F = 0$$

```
100   Print "Enter Fructose Concentration"
110   Input F
120   Print "Enter the Absorbance Value"
130   Input W
140   A=500: B=0
150   X=A-B
160   Y=X/2+B
170   G=Y-W*Exp (4.7 + 0.7 * (Y/(Y+F))) + F
180   If G > 0 then 500
190   If G < 0 then 600
195   CLS
200   If G=0 then 700
300   Print "Bad Run"
400   End
500   A=Y
510   GOTO 158
600   B=Y
```

```
 610   GOTO158
 700   Print "Fructose="; F; "Micrograms"
 710   Print "Absorbance="; W
 720   Print "Glucose="; Y; "Micrograms"
 730   M=F * .0215: N=Y * .0125
 740   Print: Print "Fructose="; M; "In Milligrams"
 750   Print "Glucose="; N;" In Milligrams"
 800   End
4900   Input Y
4910   Input F
4928   Input A
5000   G=Y-A * Exp (4.7 = 0.7 * (Y/(Y+F))) + F
5010   Print "Calc. Val.="; G; "using G="; Y
7000   G=Y-W * exp (4.7 + 0.7 (Y/(Y+F))) + F
```