

Quantitative Determination of Sugar Levels in Natural Plants of Cactus Pear (*Opuntia ficus indica*) and Votre-Coach Alimantaire Cultivated in Adigrat, North of Ethiopia

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ABSTRACT: In this work, a quantitative study for sugar concentration determination was conducted using Uv-Visible Spectroscopy. Glucose and Sucrose of three extracted plants fruit were analysis by refractometer and thin layer Chromatography before applied for quantitative determination. The TSS values recorded have a good agreement with the Sucrose standard and previously analyzed sugar in fruit and vegetables. TSS of Coach-fruit, Cactus *Opuntia* and Peel is 18Brix%, 18Brix% and 21Brix% respectively. These results have a good precision with the TSS sugar found in Apple species (18.83Brix %), Potato species (19.10%) and Grape (17.50Brix %) fruit-vegetables. The single spot observed on TLC, assay the purity of sample. Calibration models for Glucose and Sucrose determination were developed using the standard solution. Sugar concentrations were determined from single standard and calibration curve. The mean concentrations of Sucrose and Glucose in Coach-fruit, Cactus pear and Cactus peel, were 143.035mg/ml, 42.420mg/ml, and 0.943mg/ml for Sucrose; and 86.912mg/ml, 18.938mg/ml and 8.810mg/ml for Glucose determined.

KEYWORDS: sugar, carbohydrate, glucose, sucrose, cactus pear.

INTRODUCTION

Sugar is the generalized name for sweet, short-chain, soluble carbohydrates, many of which are used in food. They are carbohydrates, composed of carbon, hydrogen, and oxygen [1, 2]. These carbohydrates are especially prominent constituents of plants and usually form over one-half of the total plant substance. They serve not only as a source of available energy but also as reserve food and as structural materials. They are one of the main groups of food substances (carbohydrates, proteins, and fats) to be synthesized in the plant from simple organic substances.

The empirical composition of carbohydrates may be expressed by the formula $C_nH_{2n}O_n$. With regard to their specific chemical properties, carbohydrates may contain a potential aldehyde, -CHO, or ketone, C=O, group [3, 4].

Scientifically, *sugar* loosely refers to a number of carbohydrates, such as monosaccharides, disaccharides, or oligosaccharides. Monosaccharides are also called "simple sugars," the most important being glucose. Almost all sugars have the formula $C_nH_{2n}O_n$ (n is between 3 and 7). Glucose has the molecular formula $C_6H_{12}O_6$. The names of typical sugars end with **ose**, as in "glucose", "dextrose", and "fructose". Sometimes such words may also refer to any types of carbohydrates soluble in water. The acyclic mono- and disaccharides contain either aldehyde groups or ketone groups. These carbon-oxygen double bonds (C=O) are the reactive centers. All saccharides with more than one ring in their structure result from two or more monosaccharides joined by glycosidic bonds with the resultant loss of a molecule of water (H₂O) per bond [5].

TYPES OF SUGAR

MONOSACCHARIDES

Glucose, fructose and galactose are all simple sugars, monosaccharides, with the general formula $C_6H_{12}O_6$. They have five hydroxyl groups ($-OH$) and a carbonyl group ($C=O$) and are cyclic when dissolved in water. They each exist as several isomers with dextro- and laevo-rotatory forms that cause polarized light to diverge to the right or the left [5, 6]

Glucose, dextrose or grape sugar occurs naturally in fruits and plant juices and is the primary product of photosynthesis [7]. Most ingested carbohydrates are converted into glucose during digestion and it is the form of sugar that is transported around the bodies of animals in the bloodstream. It can be manufactured from starch by the addition of enzymes or in the presence of acids. Glucose syrup is a liquid form of glucose that is widely used in the manufacture of foodstuffs. It can be manufactured from starch by enzymatic hydrolysis [8, 9]

Fructose or fruit sugar occurs naturally in fruits, some root vegetables, cane sugar and honey and is the sweetest of the sugars. It is one of the components of sucrose or table sugar. It is used as high-fructose syrup, which is manufactured from hydrolyzed corn starch that has been processed to yield corn syrup, with enzymes then added to convert part of the glucose into fructose [10].

DISACCHARIDES

Sucrose, maltose, and lactose are all compound sugars, disaccharides, with the general formula $C_{12}H_{22}O_{11}$. They are formed by the combination of two monosaccharide molecules with the exclusion of a molecule of water [6, 11, 12].

Sucrose is found in the stems of sugar cane and roots of sugar beet. It also occurs naturally alongside fructose and glucose in other plants, in particular fruits and some roots such as carrots [6]. A molecule of sucrose is formed by the combination of a molecule of glucose with a molecule of fructose [12]

Maltose is formed during the germination of certain grains, the most notable one being barley, which is converted into malt, the source of the sugar's name. A molecule of maltose is formed by the combination of two molecules of glucose. It is less sweet than glucose, fructose or sucrose [6]. It is formed in the body during the digestion of starch by the enzyme amylase [13] and is itself broken down during digestion by the enzyme maltase [14].

Lactose is the naturally occurring sugar found in milk. A molecule of lactose is formed by the combination of a molecule of galactose with a molecule of glucose. It is broken down when consumed into its constituent parts by the enzyme lactase during digestion. Children have this enzyme but some adults no longer form it and they are unable to digest lactose [15].

FRUIT-VEGETABLES AS SOURCES OF SUGAR

Sugars are found in the tissues of most plants, but are only present in sufficient concentrations for efficient extraction in sugarcane and sugar beet [16]. Sugar occurs naturally in vegetables and fruits, as well as in some whole grains and dairy products. Naturally occurring sugars provide a healthy alternative to foods with added sugars, such as cakes, pastries, processed foods and refined breads and cereals. Fruits and vegetables have naturally low calorie and fat content and provide many important nutrients [4, 17].

CACTUS PEAR AND VOTRE-COACH ALIMANTAIRE AS SOURCES OF SUGAR

Cactus pear is an important resource for semiarid zones. Although used as forage, cactus pear fruit is primarily consumed as a fresh commodity. The processing of cactus pear fruit to obtain juices, marmalades and other kinds of processed foods in order to increase the shelf-life of the fresh fruit has been studied in recent years [18]. Similar to cactus, Votre-coach alimantaire is fruit plants consumed as food for the source of energy.

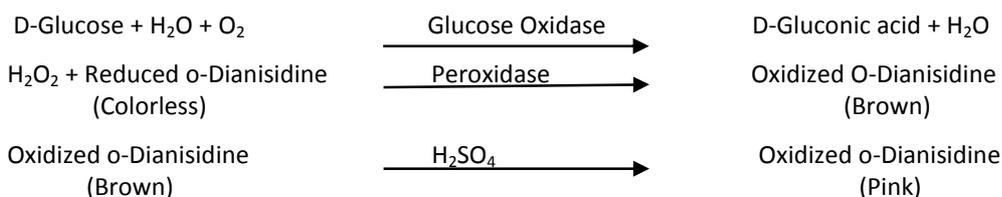
a. *Cactus pear*b. *Votri-coach alimentaire*

Cactus pear was introduced to Ethiopia between 1848 and 1920 [19, 20, and 21]. The plant is widely distributed in the arid and semi arid regions of the country; especially in eastern and southern zones of Tigray Region of Ethiopia. Over the last few decades interest in cactus pear as food and feed has increased due to its drought resistance, high biomass yield, high palatability and tolerance to salinity [22]. Cactus pear has sweetener taste. But the literature review which support the sugar content of cactus nutritional values is limit. The awareness of the human being knowledge to words the carbohydrate content of cactus and Votri-coach/Fruit de la passion is limit to explain and uses of plant as energy sources. Plant-Fruit as source of carbohydrate deals; to prove the motivation for development of carbohydrate application for society and on their use. Therefore this paper was caused full fill the gap knowledge of society have on carbohydrate content in cactus and Votri-coach alimentaire.

QUANTITATIVE DETERMINATION OF FREE SUGAR IN FRUIT JUICE AND VEGETABLE

The principle of determination of sugar concentration in Fruit Juice and Vegetable Unknown sample: - Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose Oxidase. Hydrogen peroxide reacts with o-Dianisidine in the presence of Peroxidase to form a colored product. Oxidized o-Dianisidine reacts with sulfuric acid to form a more stable colored product. The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration [23-25].

Principles:-



EXPERIMENTAL

DESCRIPTION OF STUDY AREA

The study was conducted in April, may, 2011 in Adigrat, Tigray Regional State, Ethiopia. The local farms areas of Adigrat were purposively selected. Eastern zone (Adigrat) is located between 13° 41'–13° 46' N latitude and 39° 21'–39° 34' E longitude and lies at an altitude range of 1931– 2711 masl. The zone has a monomodal rainfall (June to early September) that ranges from 140– 671 mm annually. Similarly, the annual temperature ranges between 10 – 30 °C [22].

CHEMICALS AND REAGENTS

Chemicals that were used for these investigation: ethanol (98.5%), anhydrous D-Glucose (C₆H₁₂O₆). H₂O, Sucrose (C₁₂H₂₂O₁₁), Capsule.

Chromatographic Solvents:

Acid solvent: Acetone-Acetic acid-Water, 4:1:5 v/v Mixtures. Neutral solvent: Acetone-Ethanol-Water, 40:11:19 v/v mixture.

Glucose Oxidase/Peroxidase Reagent: 500 units of Capsule were dissolved in 39.5ml of distil water and stayed below 8°C. It stable upto one month below 8°C for at least six month.

O-Dianisidine Reagent: - The pre weighed vial contains 5mg of o-Dianisidine and 2ml of Conc.HCl were dissolved into 5.0ml of ethanol.

Assay Reagent: - A reagent prepared from 0.8ml of the o-Dianisidine reagent in 39.5ml of **Glucose Oxidase reagent**.

Spray Reagents: Silver nitrate spray reagent: In 20 ml of acetone, 0.1 ml of saturated silver nitrate was added. Sodium hydroxide spray agent: Mixed 5 ml of 50% sodium hydroxide solution with 100 ml of 95% ethyl alcohol.

Glucose, Sucrose and Starch Standard Solution: - A standard solution of 1.0mg/ml in 0.1% benzoic acid.

EXTRACTION AND PURIFICATION OF SAMPLES

Source of Plant Species

Plant materials belonging to a certain investigations were selected for the sample according to their Nature and their fruit food parts; Cactus *Opuntia*, Cactus peel and Votre-coach alimantaire fruit were collected, when it appeared that mature within a variety or species might be important, sequential harvests were used.

Selected samples were sliced or dried and 16.28 (Cactus *Opuntia*/pear and Votre-coach alimantaire) , 17.84gm for Cactus peel) of raw material was placed in a 400ml Mason jar and covered with 300 ml of boiling 98.5% ethanol. Then Soxhlet-extraction apparatus were applied for extraction of each sample separately. The residue in the funnel was extracted twice again, using 300 ml of 98.5% ethanol each time. The extracts were then combined and volume reduced on water bath below at 78°C. This served to remove the alcohol. The extracts were then combined and concentrated until the extract contained sugars were have approximately the same with Sucrose and Glucose, refractive index range as indicated by refractive index measurements.

QUALITATIVE ANALYSIS OF EXTRACT PRODUCT AND PURITY OF EXTRACT SUGAR

The thin layer chromatographic were equilibrated with the solvent in the chamber and then developed for 3hours by the descending technique. The chromatograms were dried at room temperature and then spray reagents were applied for location of the sugar on the survey strips. Location of the sugars chemical constitute in sample of extracted plant; related with the reference of Sucrose in the acidic and neutral reagent thin layer chromatography is facilitated by calculating the Rf value for each sugar. Simultaneously a single spot of thin layer chromatography were observed for each sample, is indicating the purity of the extract sample.

QUANTITATIVE DETERMINATION OF SUGAR IN EXTRACT SAMPLE OF PLANTS

Sample Preparation

Method 1. Glucose Concentration from a Single Standard

The three extracted plant sugar were Votre-coach alimantaire (in 150ml of ethanol solution), Cactus *Opuntia* (in 85ml of ethanol solution) and Peel (in 75ml of ethanol solution). Cactus *Opuntia*/pear was diluted 1ml in 100ml, 5ml in 100ml and the Votre-coach alimantaire and Cactus Peel were diluted 1ml in 100ml with distilled water to bring them into the range of approximately 1 to 10mg glucose/ml.

A sample blank consisting of 0.02gm/ml of glucose (know standard of sugar) and 100ml of water and a reagent blank consisting of 1ml Reagent and 100ml of water were also prepared. After half hour the absorbance at 540nm was measured against deionized water blank. The cuvettes were measured in three trail terms.

Table 1:- Sugar determination from Single standard

Tube	Water (ml)	Sample (ml)	Glucose (ml)
Blank reagent	100	----	-----
Standard	95	-----	5
Cactus opuntia sample	99/95	1/5	-----
Cactus peel sample	99	1	-----
Votre-coach alimentaire sample	99	1	-----

$$\text{Milligram of Glucose} = \frac{\Delta A_{540} \text{ of test} * \text{mg Glucose in Standard}}{\Delta A_{540} \text{ of Standard}}$$

$$= \frac{\Delta A_{540} \text{ of test} * 0.02}{\Delta A_{540} \text{ of Standard}}$$

Where, ΔA_{540} :- Absorbance of glucose at 540nm

Multiply the mg glucose determined above by the dilution factor made in sample preparation.

Method 2. Glucose Concentration from Standard Curve

Liquids samples 2ml, 4ml of extracted sample of Cactus opuntia (150ml of ethanol solution), Cactus peel (75ml of ethanol solution) and Votre-coach alimentaire (85ml of ethanol solution) were diluted into 50ml of distilled water to bring the contraction of sugar 10-100g/ml. At zero time, the reaction were started by addition of 2ml of assay reagent to each test tube and mixed with in 2minutes; then each tube reacts exactly 30 minutes at 37 °C in water bath. At the end the reaction were stopped by addition of 2.0ml of 12N H₂SO₄ into each tube. The absorbance of each tube against the reagent blank was measured at 540 nm [24].

Table 2: - Determination of Sugar from calibration curve

Tube	Water (ml)	Sample (ml)	Sucrose or Glucose mg/l stock soln.(ml)	Sucrose Abs. at 540nm (blank reagent)	Glucose Abs. at 540nm (blank reagent)
Blank reagent	50	----	-----	0	0
Standard 1	49	-----	1	0.008	0.005
Standard 2	48	-----	2	0.016	0.009
Standard 3	47	-----	3	0.023	0.015
Standard 4	46	-----	4	0.033	0.021
Standard 5	45	-----	5	0.04	0.027
Standard 6	44	-----	6	0.048	0.033
Standard 7	43	-----	7	0.058	0.0365
Standard 8	48	-----	8	0.065	0.041
Standard 9	41	-----	9	0.071	0.047
Standard 10	40	-----	10	0.08	0.052
Cactus opuntia sample	48/46	2/4	-----	0.090/0.182	0.026/0.049
Cactus peel sample	48/46	2/4	-----	0.0041/0.079	0.023/0.048
Votre-coach alimentaire	48/46	2/4	-----	0.583/0.988	0.029/0.040

RECOVERY TEST OF REAGENTS AND METHOD

Recovery of added Sucrose was nearly 99.87% (Tab. 3).

Table 3: Recovery Test of Sucrose

Results (mg/l)	Sucrose (mg/ml)	Added Sucrose (mg/ml)	540nm concentration measured (mg/ml)	Theoretical concentration(mg/ml)	% Recovery
1.00	0.50	1.50	1.50	1.50	100
1.00	1.00	1.99	1.99	2.00	99.5
1.00	2.00	2.99	2.99	3.00	99.66
1.00	3.00	4.00	4.00	4.00	100.00
1.00	4.00	5.01	5.01	5.00	100.2

RESULTS AND DISCUSSION

MOISTURE CONTENT OF PLANT SPECIES

The moisture content is expressed as mass fraction, in percent, of the mass of the initial sample. A 350gm, 125gm and 350gm of fresh mature nutrient parts of Cactus opuntia, Votre-coach alimantaire and cactus peel were respectively dried with in air hot oven until all of the moisture was evaporated, from the plants. The 16.28gm, 16.28gm and 17.84gm of dry weight Votre-coach alimantaire, Cactus opuntia and Cactus peel were obtained respectively. These results are indicating; the moisture content of Votre-coach alimantaire 86.97%, 95.3% for Cactus opuntia, when moisture content of cactus peel is 94.9%. Cactus Opuntia is shows the highest water content, when cactus peel is the lowest one.

DETERMINATION OF TOTAL SOLUBLE SOLIDS AND R_f VALUE OF EXTRACTED SAMPLE

During the development of the flesh of a fruit, in many species, nutrients are deposited as starch, which during the ripening process is transformed into sugars. The progression of the ripening process leads to increasing sugar levels. This sugar concentrations' concentrated in sample of plants or total content of soluble solids (TSS) or sugar in a fruit were recorded by refractometer (Table 3). The method is especially suitable for ripe and juicy fruit, with significant sugar content, as the determination of TSS is based on the capacity of sugars in a juice to deviate light.

The results, obtained from chromatography are strongly recommended, the chemical composition of sugar extracted from Cactus Opuntia and Votre-coach alimantaire is similar to the known standard sugar of sucrose and glucose.

Table 3: - R_f value of sugar samples in acid and neutral solvent and Refractometer

Sugar	Rf value		Refractometer of TSS (Brix 0.1%)
	Acid Solvent	Neutral Solvent	
Known Sucrose	0.92	0.75	16
Coach-fruit	0.8	0.695	18
Cactus opuntia	0.82	0.786	18
Cactus Peel	0.846	0.717	21

The results of refractometer index in Table 3, indicate exceptional the TSS of cactus peel, 21%Brix, the result obtained at Cactus opuntia (18% Brix) and Votre-coach alimantaire fruit (18% Brix) is approximately have a good agreement with the Sucrose (16% Brix).

DETERMINATION OF SUGAR

a. Determination of glucose from a single standard

The measured amount of glucose obtained by the assay, from single standard determination sugar method allocates the Sucrose concentration in extract plants are 102. 51mg/ml, 33mg/ml, 0.47mg/ml in Votre-coach alimantaire fruit, Cactus opuntia and cactus peel respectively. The sugar content of fruit and plants leaf consists mainly of glucose, fructose and sucrose and the profile of each of these depends strongly on the cultivar and region in which the fruit is grown.

b. Sucrose and Glucose Concentration from Standard Curve

Votre-coach alimantaire is marketed as a high energy vegetation to rapidly provide energy enhance after intense physical activity. This is reflected in the high level of sucrose and glucose which provides a ready source of carbohydrate that can be easily metabolized. The measured amount of glucose obtained in Cactus peel by the assay was found to be less than half of Cactus opuntia under the same environment. The mean concentration of Sucrose and Glucose recorded in Votre-coach alimantaire fruit, Cactus opuntia and Cactus peel; 143.035mg/ml, 42.420mg/ml and 0.0943 concentration of Sucrose, when 86.912mg/ml, 18.938 and 8.810mg/ml concentration of Glucose were obtained respectively. The sugar content of each fruit and plant species consists mainly of glucose, fructose and sucrose and the profile of each of these depends strongly on the cultivar and region in which the fruit is grown.

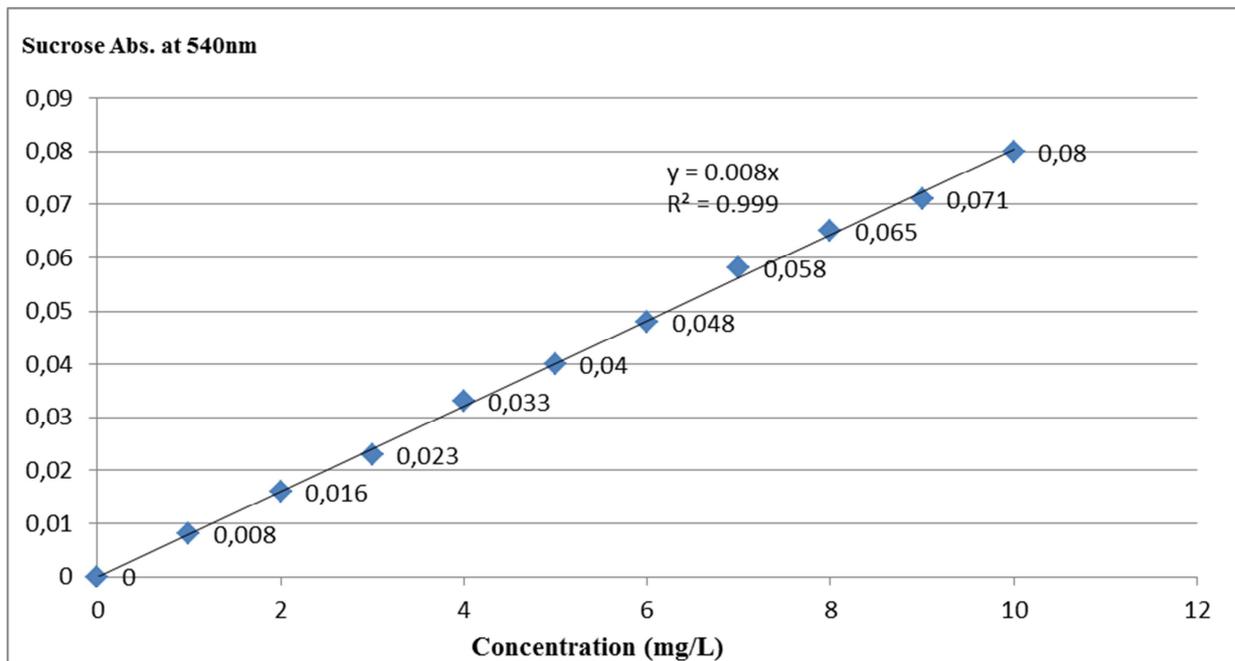


Figure 1:- Standard curve for the determination of glucose

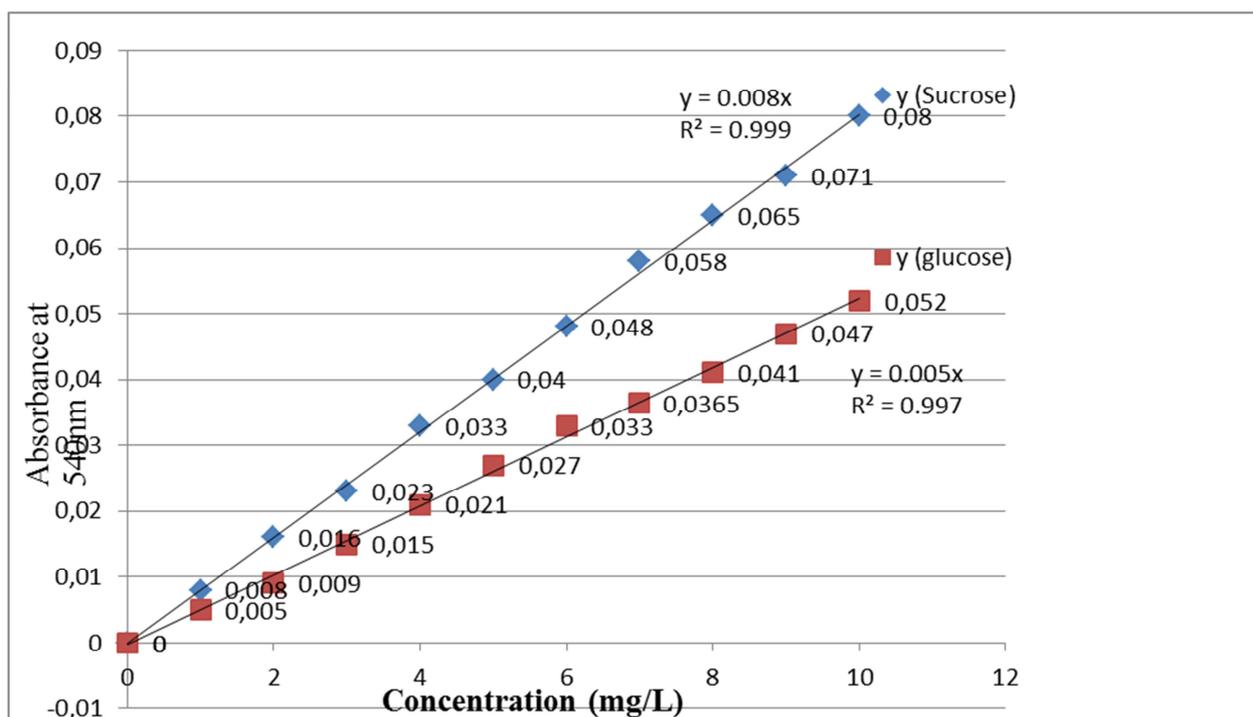


Figure 2:- Standard curve for the determination of Sucrose and glucose simultaneously originate from the same origin.

Table 4:- Concentration of Sucrose and Glucose in Votre-coach alimantaire, Cactus *Opuntia* and Cactus peel

Plants	volume of Sample	Sucrose Concentration(mg/ml)	Glucose Concentration(mg/ml)
Votre-coach A.	2ml	154.850	88.825
	4ml	131.220	85.000
	Average	143.035	86.912
Cactus <i>Opuntia</i> /pear	2ml	42.185	19.500
	4ml	42.656	18.375
	Average	42.420	18.938
Cactus peel,	2ml	0.961	8.625
	4ml	0.926	9.000
	Average	0.943	8.810

The sugar content of Votre Coach Alimantaire and cactus consists mainly of glucose and sucrose and the profile of each of these depends strongly on the nature and maturity age in which the fruit is grown. The glucose content of Votre coach alimantaire can range of 85.00 to 88.825 mg/ml, when the ranges of sucrose content can 131.00 to 154.850mg/ml in analyzed plant fruit. The contents of glucose and sucrose, 42.185 to 42.656mg/ml and 18.375 to 18.938mg/ml respectively were recorded in cactus pear fruit. The minimum glucose and sucrose were recorded in Cactus peel related to the remains two.

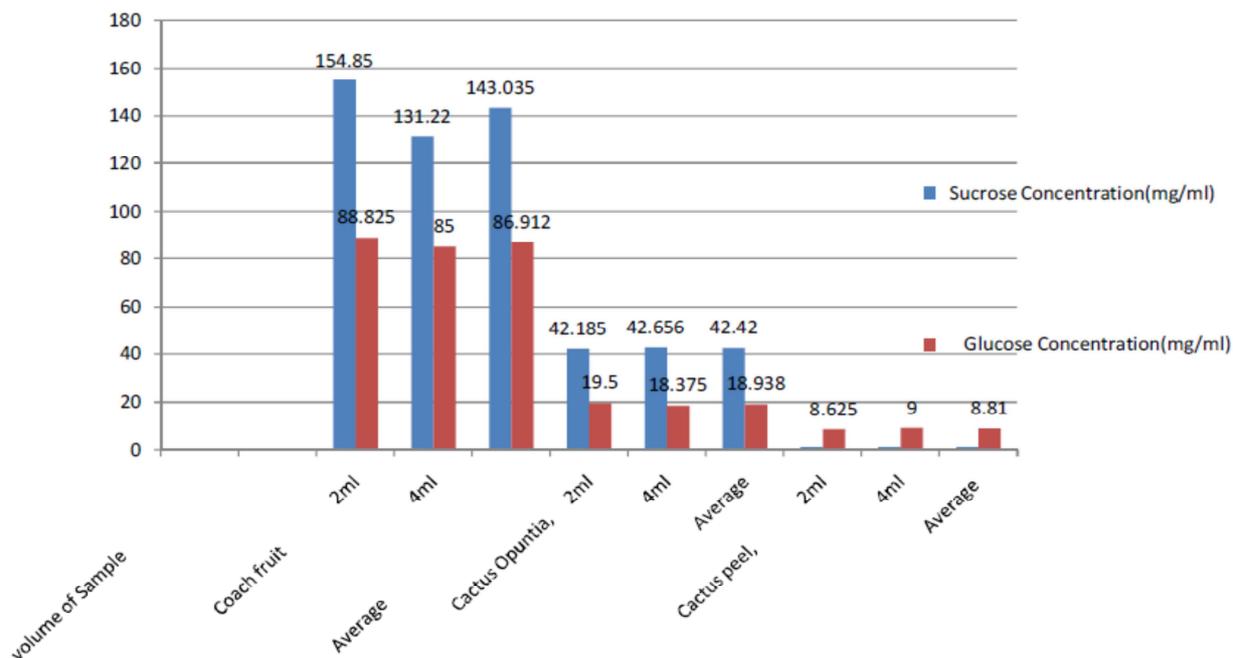


Figure 3:- The concentration of Sucrose and Glucose respectively recorded in Sample of fruit- vegetable plants

CONCLUSION AND RECOMMENDATION

The presented method allows for measurements of the content of glucose and sucrose in starch rich foods such as fruit – vegetables and maturing plants consumed as food sources. The method is quick, easy and reliable. The extraction of plants nutrient by Soxhlet- extraction method one of the easiest method applied for extraction of Votre-coach alimentaire and Cactus plants. However, in the analyses, the Glucose and Sucrose needs to be precipitated using ethanol. The study showed that the maximum concentration of Sugar were determined in Votre-coach alimentaire Fruit, when lowest value was determine from Cactus Peel. Approximately more than twice values of Sucrose are found in each plant-fruit comparing to Glucose.

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