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A CRITICAL APPRAISAL OF METHODS OF SUCROSE ANALYSIS

IN SUGAR BEETS

by

Homer M. Lebaron

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Agronomy

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Gratitude is extended to my wife, Venita, whose patience and sacrifice made this study possible.

Homer M. Lebaron

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INTRODUCTION

Although the parent of the present sugar beet was probably known and used before the building of the pyramid of Cheops (22), it was as recent as 210 years ago that Marggraf obtained the first crystallized sucrose from the root. It was 40 years later before Achard claimed its potentialities as an efficient industry. The product rapidly passed from the status of a luxury to a vital necessity, until today sugar beet production receives some protection or preference in every country where sugar beets are grown.

Throughout its 174-year history it has probably been beset with a greater variety of problems and more failures than any other industry before or since. One of the greatest problems has been the actual means of sucrose extraction and determination.

The determination of sucrose in sugar beets is the most important function in a beet sugar factory laboratory (3). This value is necessary as the basis for calculations of sugar yields and losses, for fixing the value of beets in factories where these are bought on the basis of their sugar content and for other purposes. Sugar content determinations are also of vital importance to the plant breeder, soil scientist, plant physiologist, and other researchers concerned with sugar beet analysis.

Values of such importance should be determined by methods of corresponding accuracy. However, in 1927 Stanek and Vondrak (3) stated that there is no routine method as yet which permits the determination of the sugar in the beet to within an accuracy of 0.1 per cent. It is doubtful

that the fundamental accuracy of the saccharimetry methods has improved much since this time.

Numerous methods of sucrose analysis are used and are being recommended for use both in sugar beet factory and research laboratories. Although most methods now used are based on the principle of aqueous digestion and single polarimetry, there are wide variations in actual procedures. The available literature is deficient in adequate comparative studies into this problem. It would be of considerable value to researchers in sugar beet production and analysis to know the relative accuracy and precision of these methods and the effects of the several variables now prevalent.

REVIEW OF LITERATURE

Methods of sucrose analysis

Sucrose analysis of sugar beets has undergone considerable change and variations in the past (6).

A brief history of sucrose extraction and analysis will indicate some of the problems involved and serve as background for the development of present methods.

The determination of sucrose in sugar beets by polarization of the expressed juice was formerly quite common (6, p. 345). It has been almost entirely abandoned because of unavoidable errors and problems involved.

The method most accurate in principle for sucrose analysis in beets is extraction (6, p. 353). The solvent most generally used is 90 per cent ethyl alcohol. The sugar is washed from the pulp by reflux distillation and the extract is made up to volume and polarized. The principal sources of error are the effect of alcohol on the polarization of non-sugars and the effects of excess lead acetate and of the prolonged heating on the rotation of sugar and non-sugars. The alcohol extraction method does not presently occupy the position as a standard procedure that it formerly did in saccharimetry. It was not best from the practical standpoint because of the long period of time necessary for extraction, and because of the fragile nature of the extraction apparatus (6, p. 359).

The process of percolation with water as the solvent has been used (6, p. 357). The principal sources of error with water are incomplete extraction, inversion of sucrose through natural acidity of the pulp, and the solution of optically active gums, hemicelluloses, and pectic acid.

For the rapid determination of sucrose in sugar beets and for routine analysis, one of the numerous digestion processes is usually followed in present research. In principle, the digestion method is a combination of the extraction and juice-expression methods, in which a weighed amount of pulp is digested with a large excess of alcohol or water. After the complete diffusion of the sucrose through the solvent, the solution is brought to volume, allowing for the space occupied by the marc, and then filtered and polarized.

The use of alcohol as the solvent has been generally abandoned in favor of aqueous digestion because of unreliable results and because it is too expensive for routine work.

The aqueous methods of digestion may be subdivided into three classes (6, p. 363): (1) methods in which the normal weight of pulp is made up with water to a definite volume (Pellet's method and modifications); (2) methods in which a definite volume of water is added from a pipette to a normal weight of pulp (Sachs-LeDocte's method and modifications); and (3) methods in which pulp and water are mixed in definite proportions, but a normal weight is not required (Kruger's method).

Although they have been variously modified, the basic methods of Pellet and Sachs-LeDocte are used almost exclusively by factory and research laboratories for routine sucrose analysis in all countries. The Kruger method has never been very popular and has been generally abandoned (6, p.371) because of the inconvenience and confusion of using pipettes differing in volume in the same laboratory.

Credit is due to Pellet (20) for championing the method of hot water digestion which is usually given his name (6, p. 363). It was devised

by him in 1887 and at about the same time by Herles (6, p. 363). Although it has become variously modified and there is still not uniformity in detailed procedures, it is the generally accepted method in all countries for determining the percentage of sucrose in cossettes, for sugar factory control analysis, and for other research. It does not require pulp of extreme fineness.

S. J. Osborn (20) has made a study of several of the details in the procedure which he found to be important. He suggests that half-normal (26 grams of pulp per 200 ml. of solution) concentration is preferable to full normal concentration to insure complete diffusion and extraction.

Variations of digestion temperature seemed to be unimportant within reasonable limits, for the pulp used. The upper limit of the bath temperature should not be more than 80° C. in order to prevent decomposition of pectins, hemicelluloses, and other marc constituents. All air must be removed by swirling and applying a vacuum, especially when rasped or finely divided pulp is used. Periodic swirling is allowed by carrying out the initial digestion with an incompletely filled flask. A supplementary digestion period of 10 minutes is recommended after bringing the flask approximately to volume with hot water. This will insure equilibrium of concentration.

Pellet (6, p. 356) also devised a cold water digestion process. It has been entirely superseded by the Sachs-LeDocte method because of the danger of incomplete extraction in the flask and because of the greater inconvenience of the pipette method.

The main objection to the Pellet method in principle is the occlusion of air bubbles by the pulp and the uncertainty of knowing whether such

bubbles are completely absent when bringing to volume. To overcome this problem, Kaiser and Lowenberg (6, p. 356) in 1892 proposed to add to the normal weight of pulp a constant volume of water and lead subacetate solution so that the final estimated volume of solution, regardless of occluded air or insoluble marc, is always 100 ml. This idea was further modified by Sachs and LeDocte (6, p. 365; 3) in 1895 with the total volume increased to 200 ml. to insure complete extraction and perfect admixture, and to obtain more filtrate for polarization. They calculated that the volume of water and lead subacetate solution to be added was 177 ml. The original 177 ml. pipette is still the one most widely used, but for greatest accuracy the volume must be adjusted to suit the beets grown in a particular country or district.

Tare laboratories in the United States almost invariably use the Sachs-LeDocte method of water digestion for sugar analysis (15, p. 95), but occasionally checks are made against Pellet digestions. Although a finer divided pulp is required, it is faster and more convenient for routine analysis.

Genotelle (16) has suggested a rapid Sachs-LeDocte modification using a high-speed comminuting apparatus for extraction. The Waring Blendor extractions have also shown good comparison and agreement with the hot Sachs-LeDocte method. Only 1 minute blending time is required. Moore and Hallbeck (16) feel that this modification offers an opportunity for considerable simplification of tare laboratory methods where finely rasped pulp is used.

The trend in modern tare laboratory operation is toward mechanized and high speed equipment and instrumentation. In the control laboratory emphasis is placed on accuracy and precision.

The hot water digestion methods are considered more reliable for controlling losses in the factory. The quicker cold digestion methods should be used only if apparatus is available for securing pulp of extreme fineness, and are of greatest value when rapidity rather than highest accuracy is required.

Sources of error in aqueous digestion methods

There are two main sources of error in the aqueous digestion methods in general (14).

Errors due to marc determinations. The flask volumes or amounts of lead water adopted to correct for the volume of marc and lead precipitate are average values and individual beets may differ appreciably from the average, especially if the beets are unripe, wilted or watery (6, p. 371). There is no complete agreement on a standard average volume allowance for marc and lead precipitate, and possibly there should be none because of the variations of beets in different locations. Beets from the same soil may vary considerably in marc content from season to season and even within the same season. Marc determinations on 50 beets in 1956 from the same plot (25 ft. x 5 ft.) varied from 3.94 to 6.34 per cent, with an average of 4.944 per cent (2). If we assume the density of marc to be 1.13 gms/cc these variations represent from 0.91 cc to 1.46 cc with an average volume of 1.14 cc for a normal weight of pulp. Therefore it has been left to individual countries or districts to decide their own volume correction (6, p. 362).

However, the problem is complicated by the general lack of agreement on the definition of marc and the method used for its measures. Bachler (3) defines marc as the more or less "water-soluble," sugar-free, porous,

cellulosic skeleton which gives body to the beet, and in which the walls and some of the cells are saturated with colloidal or imbibition water. (It seems that this should be "water-insoluble" to be consistent with Bachler's definition of marc.)

Browne and Zerban point out that the general definition of marc as the water-insoluble portion of the beet is inadequate since the type and quantity of dissolved material varies with the temperature and nature of the solvent used, with the length and manner of treatment, with the general condition of the beet, with the particle size of the beet pulp, with the ratio of solvent to pulp, and with methods of procedure (6, p. 347). They state that a temperature of about 65° C. is required to destroy the protoplasm and to facilitate the extraction of the soluble substances. But at this temperature the hemicelluloses and the pectins are attacked and converted into water-soluble substances. If alcohol is used for the extraction, the albumen is coagulated and a part of the salts is not extracted.

The composition of beet marc, according to Smolenski, is about 22 per cent cellulose, 73 per cent pectin, and 5 per cent albuminous substances (6, p. 352). All these hold a certain amount of water in the imbibed or colloid form.

By the usual analytical procedures for more determinations the marc is weighed bone dry. Using this for the first of all digestion methods (the alcohol digestion), Rapp and Degener (6, p. 360) in 1882 assigned to the normal weight of marc a volume of 0.6 cc. This value was later accepted by other researchers for their aqueous methods, which led to the general use of 200.6 ml. flasks for the Pellet method and 177 ml.

pipettes for the Sachs-LeDocte method (3). This volume has been widely criticized during the past 40 years on the grounds that the anhydrous marc determination does not take into account the natural state of turgescence of marc or the presence of sugar-free imbibition water as it exists in the beet. It also disregards the lead precipitate formed in clarification.

Early tests to determine the volume occupied by hydrated marc in the normal weight of beet gave values from 2.0 to 2.5 ml. (6, p. 361; 3). Later investigations showed that the volume of marc hydrate in the presence of lead subacetate decreases to about 1 ml. and excess lead causes further shrinkage (6, p. 362). It was concluded that the lead subacetate causes the marc hydrate to shrink by withdrawing colloidal water. It is now generally conceded that the old volume correction of 0.6 cc is too low, but there is still disagreement regarding methods of marc determination and whether the aim is to find the pure marc hydrate or the marc hydrate plus lead precipitate.

Bachler (3) prefers the measurement of marc hydrate (marc in its original state of turgescence) as the volume correction for the Sachs-LeDocte method. This he has determined to be 2.31 cc in a normal weight of average beets from Southern California. Therefore he recommends using a pipette of 179.1 cc. He feels that two methods eventually will evolve, one which will determine the pure marc hydrate, a knowledge of which is important for the Sachs-LeDocte method analysis, and another which will be suitable for the determination of the volume of marc hydrate plus lead precipitate, which is important for the Pellet method.

It seems that the problem of which volume correction to use should

not depend on whether the Sachs-LeDocte method or the Pellet method is the procedure followed. The question is whether or not the colloidal water withdrawn from the marc hydrate when lead subacetate is added should be considered part of the juice or if it is still to be considered as marc, since if there is agreement as to what is marc and what is juice, the volume correction by either process is identical.

Osborne (3, 19, 21) in 1923 was the first to claim 1.0 ml. for the average volume of marc plus lead precipitate. This has come to be the most generally accepted volume correction where the Pellet method is employed. He noted that while there is considerable variation in the value obtained, too great an importance should not be attached to the absolute accuracy of the calculated marc volumes. The accuracy of polariscopic observations is not sufficient to establish the desired value with precision.

Hungerford and Koontz (13) made a study to compare the Sachs-LeDocte methods (both hot and cold) and the Pellet method with a Soxhlet extraction method developed by Hartmann. Assuming that the Soxhlet extraction procedure gives the correct content of sugar in beets, they conclude that the pipette volume for the Sachs-LeDocte hot digestion should be 178.4 ml. while the flask volume for the Pellet hot digestion should be 201.6 ml. However, if the Sachs-LeDocte cold digestion is used, they recommend the 177 ml. pipette in order to obtain the same average result as with the 178.4 ml. pipette for the hot digestion.

One should not be so concerned about a volume correction factor to allow for hot digestion compared to cold digestion; of more fundamental importance is how variable is the increase by hot digestion with beets

from different environments and what are the causes.

Errors due to impurities. All methods of simple polarization for the determination of sucrose are based on the assumption that the only optically active constituent in the digestion juice is sucrose, and that its normal rotation of polarized light is unaltered by accompanying impurities. This assumption is rarely if ever justified in practice. No complete analysis of beet juices is known and their composition is far from constant (15, p. 177).

Normal beets contain small quantities of invert sugar, raffinose, amino acids, and other optically active substances. Their normal rotation may also be greatly affected by the lead subacetate used for clarification and by the presence of other impurities. The combined effect of these influences may be an appreciable plus or minus error, or the correct result may be obtained through mutual compensation.

The solvent used, the temperature of the bath, the length of digestion period, the pulp particle size, the condition of the sugar beets, the efficiency of clarification, and perhaps other factors all contribute to the total effect on the extraction and polarization of the many nonsugars present.

Browne (5) and others (6, p. 322; 17) report that when invert sugar is present, there occurs an increase in dextrorotation due to the precipitation of levo-rotating fructose or the formation of soluble dextro-rotatory lead fructosate by the basic lead salts. However, glucose and fructose are easily oxidized and decomposed into acids and colored products, so they are seldom found in appreciable concentration (15, p. 181).

Raffinose is chemically rather unreactive and is strongly

dextrorotatory (+ 124°) compared to sucrose (+ 66.5°). Maclay, et al. (14) found that California sugar beets generally were too low in raffinose to necessitate consideration, but suggest that a correction factor might become necessary if 0.05 per cent raffinose were present. The raffinose content of 50 Utah sugar beets that have recently been analyzed ranges from 0.09 per cent to 0.31 per cent, with an average of 0.175 per cent. These concentrations would induce a plus error of 0.17 per cent to 0.58 per cent with an average of 0.33 per cent sucrose.

The number and types of amino acids and their concentration vary greatly between beets. As many as 18 amino acids have been indicated in certain beet juice samples by paper chromatography (10). All of these amino acids are optically active except glycine (24, Vol. I, p. 322) and their specific rotation is generally altered by the basic lead acetate salts. Although the amount of glutamic acid in the sugar beet may vary from .01 to over 1.0 per cent (26), the majority of the amino-nitrogen fraction of the beet is usually in this form. Asparagine usually comprises most of the remainder. In the presence of lead subacetate, the optical activity of glutamine shifts from slightly dextrorotatory to slightly levorotatory, while the specific rotation of asparagine changes from -504° to strongly dextrorotatory (17; 6, p. 372). Maclay, et al. (14) found that the dextrorotatory amino acids were practically nullified by the levorotatory acids in a test made on artificially prepared solutions of amino acids. The result would depend on types and concentrations of amino acids present and the net effect on polarization would be expected to vary considerably.

If the beets are unripe, frost-bitten, diseased, or otherwise

abnormal, or if the digestion is carried out at too high a temperature (above 85°C.), high polarizing dextrorotatory gums, pectins and hemicelluloses will be extracted (6, p. 373). Pellet (6, p. 372; 17) claims that if sufficient lead subacetate is used, these compounds are completely precipitated, but McGinnis states that pectins are difficult to clarify with lead acetate (15, p. 185). Hungerford and Koontz (13) report an average increase of more than 0.1 per cent sugar from the Sachs-LeDocte hot digestion compared to the cold digestion.

Other substances in the juice such as polyphenols, tyrosine, saponins, nitrogenous bases, ammonia, iron, aluminum, sodium, and potassium may interfere with sucrose analysis (15, p. 185). Honig claims that inactive substances such as salts of sodium and potassium decrease the rotation of sucrose (12, p. 69). However, he does not cite evidence to support this claim.

Most investigators agree that while the specific rotation of sucrose is not appreciably altered by lead subacetate when recommended amounts are used, its dextrorotation will increase considerably when an excess of the lead reagent is used (7, p. 323; 14).

Osborn (20) states that the destruction of sucrose during digestion without lead acetate added is too small to be of consequence. Since there is no determinable difference whether the lead is added before or after digestion, the addition of lead after digestion has been incorporated in the standard method of analysis of the Great Western Sugar Company.

However, Hungerford and Kunz (13) report that when the lead solution was added before digestion by the Pellet method, slightly higher average results were obtained.

Bachler (6, p. 368) suggests clarifying with Horne's dry lead subacetate after the digestion of pulp with distilled water. By removing a small portion of the digestion juice before adding the dry lead, it is possible to determine both the soluble solids and the sucrose content from the same sample.

In 1935 Browne (5) reported that when basic lead acetate was used for clarification, higher sucrose determinations resulted by direct polarization; however, dry lead subacetate only gave an average increase of about 60 per cent of that obtained when lead subacetate solution was used. Polarization figures found by the dry lead method are known to be lower than similar figures found by the wet methods, but the value and entire cause of this difference has not been satisfactorily determined (9). Research indicates that dry lead subacetate clarification reduces the volume or precipitate error and removes more of the reducing sugars than the lead subacetate solution, but there is greater susceptibility to error due to excess of lead (5).

The selection of an appropriate clarifying agent is one of the most important operations of saccharimetry. Rapid filtration and brightness of clarification are factors which must be considered, as well as minimum degree of error. The smallest possible quantity of clarifying agent should be used. The use of lead subacetate has become almost universal in sugar beet saccharimetry in spite of its inherent errors and shortcomings.

Preparation of sample

In the analysis of sucrose in sugar beets, special stress must be laid upon the correctness of sample. Accuracy in analytical details is obviously of no value unless the portion of pulp weighed out for

examination is a true and representative sample of the entire beet or lot of beets in question.

Some understanding of the morphology of the sugar beet root is necessary in obtaining true samplings. The percentage of sugar and purity is low in the leaves and high in the mature root. The crown, the transitional zone between root and leaves, has some characteristics of both. It has a considerable concentration of nutrients on the way to the leaves, sugar on its way to the root, and nonsugar products similar to those retained in the leaves. As we go down the root from the crown, the percent sugar and purity increases rapidly and is highest slightly below the point of maximum girth, decreasing gradually again to the root tip (15, p. 37). Examination of a horizontal cross-section of the root also shows considerable variation in sugar content and purity from center to skin. The core of the beet is relatively dark and woody, and is surrounded with wings of white, crisp substance alternating with dark, woody rings. Sugar is stored in the white egg-shaped cells. The darker core and rings contain bundles of fibrous tubes or ducts, through which the water and nutrients from the soil pass on their way to the leaves. Since the darker rings are in greatest number in the core and near the skin, it is natural that these areas are lower in sugar and purity (15, p. 38). Chemical analysis will bear this out and show that there is a gradual increase in sucrose content and purity from the core to the outside one-eighth inch rind layer where they drop sharply (8). It is evident, therefore, that in order to obtain a representative sample where we only prepare a portion of the beet, as is the case in most research and tare laboratory analyses, the pulp taken must represent the entire root from core to skin and from

crown to root tip and must also be in proportion to the amount or size of beet throughout. This could be done by cutting the beet in quarters longitudinally through the center of the core or by removing a wedge-shaped segment, the edge of which coincides with the axis of the beet. Most of the numerous rasps, shredders, graters, and other macerating machines have been designed with this objective. If a hot water digestion method is to be used for analysis, the cellular tissue does not need to be disintegrated, but a meat chopper, such as the Enterprise chopper No. 41 is recommended (20).

With any type of rasp or chopper the pulp sample as obtained is not uniform and must be thoroughly mixed before weighing. Mixing is often done by hand, but a mechanical mixer of the egg-beater type has been devised which gives better and faster mixing, and at the same time eliminates a disagreeable job and most of the labor and human element (23).

Some sampling has been done where the entire beet or lot of beets has been reduced to pulp, thoroughly mixed, and one or more samples are taken and analyzed. The Hobart food cutter (model T215) has been very satisfactory for this procedure (1). This method has important advantages over the rasp, the meat grinder, and other methods of sample preparation. If the pulp is properly mixed, representative samples are obtained since the entire beet is processed. The coarseness of pulp can be varied within certain limits by length of cutting time. The pulp particle size is quite uniform at any one time. The beet material is not subjected to great pressure which causes pulp and juice separation.

The principal objections to the rasp for sample preparation are:

(1) decomposition and evaporation are rapid; (2) separation of juice and

pulp; (3) difficulty in obtaining a representative sample; (4) optically active substances and other juice impurities are more freely liberated during digestion; and (5) sample obtained is subject to the condition of the beet, size of the beet, shape of the beet, and the condition of the rasp.

Owing to the liability of sugar beet pulp (especially when rasped) to change in composition through evaporation of water and through decomposition by the action of enzymes and microorganisms, it is important that analysis be begun as soon as possible after sample preparation. Changes in composition due to enzymes may consist in an inversion of sucrose, in a modification of gums, hemicelluloses, etc., or in loss of sugars through respiration. Therefore, it is impossible to preserve untreated sugar beet pulp for any length of time without change in composition, although the rate of change may be greatly retarded by cold storage. Heating the sample before storage will destroy enzymes but may cause inversion, etc. Freezing may suspend enzyme action for the time, but may incite other changes. Rapid cooling to 3° C. or lower will preserve sugar beet pulp for some time, but samples must be analyzed immediately after thawing. The research results reported by Gormany (7) indicate that the deep freezing of pulped beet samples for later sucrose analysis is reliable.

Osborn (20) found, in agreement with other investigations (4), that coarse unfrozen pulp such as is obtained from a meat grinder shows an adsorption of sugar effect. Finely rasped pulp shows no determinable adsorption. However, the coarse pulp also tends to adsorb some colloidal water. The net effect of these two adsorption phenomena on sucrose analysis is not known and they may largely counteract each other.

METHODS OF PROCEDURE

Experimental design

Several variables in the aqueous digestion methods of sucrose analysis by single saccharimetry were evaluated. The principal experiment was designed as a $2 \times 3 \times 4 \times 4 \times 4$ split-plot factorial in which effects of freezing, three sucrose or purity levels, two basic methods each at two temperature levels, four degrees of coarseness, and four lengths of digestion time were studied. The treatments were replicated three times.

The effect of freezing was studied by analyzing samples freshly prepared from unfrozen beets and comparing these data with results obtained from frozen samples from the same beets.

The effect of three levels of sucrose content was studied by varying the nitrogen content of the soil. The variations in process were as follows:

P₁---Sachs-LeDocte cold water digestion (20° C.).

P₂---Sachs-LeDocte hot water digestion (55° C.).

P₃---Pellet hot water digestion (55° C.).

P₄---Pellet hot water digestion (80° C.).

The degrees of coarseness of pulp sample were obtained as follows:

C₁---chopped in the Hobart food cutter (Model T215) for 1 minute.

C₂---cossetted with a Mouli salad maker, and cossettes were ground in an Enterprise meat grinder with one-quarter inch perforations.

C₃---chopped in the Hobart food cutter for 10 minutes.

C₄---rasped on a Keil-Dolle segment rasp.

The four digestion periods were studied as follows:

T₁---15 minutes.

T₂---30 minutes.

T₃---1 hour.

T₄---2 hours.

Field experiment

Since the sucrose content and purity of sugar beets generally decrease as nitrogen content of the soil increases (11, 26), the sucrose or purity levels were induced in the same field of beets by varying the nitrogen levels. Each field replication consisted of three levels of nitrogen.

N₀---no nitrogen

N₁---80 pounds of elemental nitrogen per acre, applied in bands near planting time.

N₂---250 pounds of elemental nitrogen per acre; one-half side-dressed near planting time and one-half side-dressed on July 20.

All of the nitrogen was applied in the form of ammonium sulphate in bands approximately 2 inches deep and 3 inches from each side of each row. The applications were made with a Planet Jr. seeder.

Each plot was 35 feet long by 11 2/3 feet wide, consisting of seven rows, 20 inches apart. There were 6-foot alleyways between plots at the head and foot of each plot.

All plots received equal applications of water. Irrigations were adequate to prevent any deficiency even on the high N plots. Eight irrigations were given as needed (based on general observations) throughout the growing period from June 11 to September 26. Approximately 2 to 3 inches of water were applied per irrigation. In addition, 4.71 inches of precipitation fell during the growing season.

The beets were harvested on November 12 and 13. At the time of harvest, a 5-foot length was discarded from each end of each plot. Only the center three rows were sampled. This left approximately 75 beets in the three 25-foot rows. Thirty beets were randomly selected from these in each plot. The tops were removed with a knife. Care was taken to leave as much of the crown as possible by cutting off all the petioles just below the growth initials. As soon as the beets were topped they were placed in heavy insulated paper bags, tied and stored. The temperature during harvest was below freezing but the beets were not frost-bitten.

Sample preparation

When all the steps in sample preparation and laboratory procedures had been worked out and trial runs had been made, the 30 beets from one of the three nitrogen levels were taken from the first replication and transported to the sample processing laboratory. There they were thoroughly cleaned with water and bristle brushes, and the root tips were cut back to the diameter of approximately 1 inch. A wedge-shaped portion was removed from each beet by a Keil-Dolle segment rasp. Figure 1 shows the type of segment removed. Each beet was carefully cut into four equal quarters in line with and through the center of the beet as in figure 1. The quarter from each beet having the segment removed by the rasp was discarded. One of the three remaining quarters from each beet was taken and these were bulked in three equal lots, each of which was processed as outlined.

All the quarters in one lot were placed in a Hobart food cutter (Model T215) and chopped for exactly 1 minute. A second lot was placed in the cutter and chopped for exactly 10 minutes. The third batch was

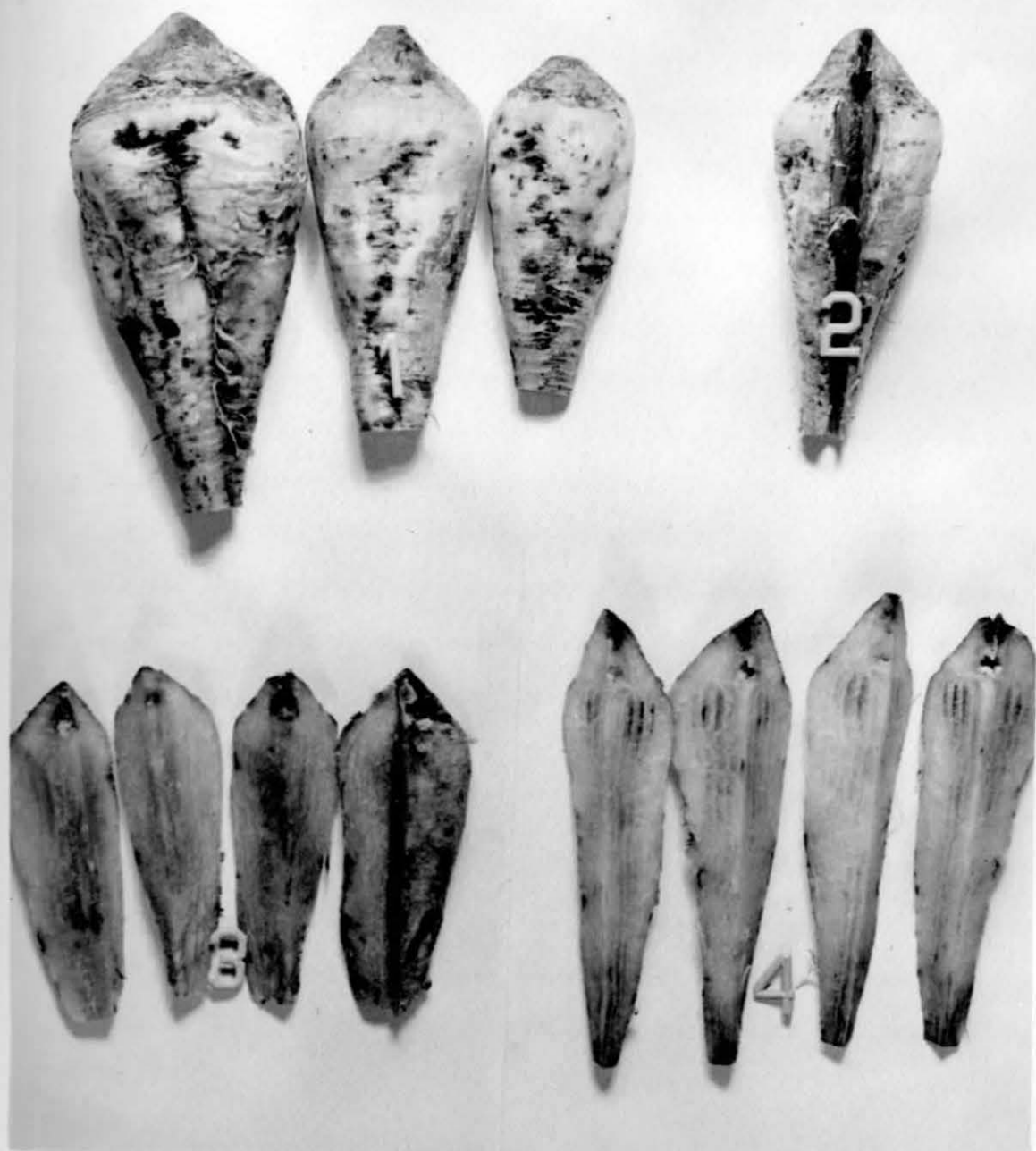


Figure 1. Sugar beets and method of quartering. (1) General condition of beets before sampling. (2) Segment removed by the rasp. (3) Division of beet after rasping. The quarter on the right is discarded. (4) Quartering of a beet in line with its axis.

first run through a hand food shredder to approximate the condition of factory cossettes; these were then run through an Enterprise meat grinder fitted with a plate having one-fourth inch perforations. All four of the sample preparations were thoroughly mixed by hand. The appearance and general condition of the four pulp preparations may be seen in figure 2. An adequate number of samples of all four degrees of coarseness were quickly weighed out at approximately 30 grams each on square sheets of aluminum foil. Each sample was wrapped, sealed, tagged and placed in dry ice. When all samples for future study were prepared, they were rushed to the locker and were frozen as quickly as possible.

Part of the four sample preparations was covered to prevent evaporation and taken to the analysis laboratory where 16 samples of each were quickly and accurately weighed out to 26.00 grams. Periodically during the weighing process the rasped material was mixed as a precaution against pulp and juice separation.

Sucrose and purity analysis

Eight of the 16 samples from C₁, C₂, C₃, and C₄ were weighed directly into 201.0 ml. Kohlrausch flasks for the Pellet digestion method and the remaining eight were weighed onto a tared onion skin paper 4 inches square for the Sachs-LeDocte process. Both weighing procedures are shown in figure 3.

Pellet method

The determination of sucrose in sugar beets by hot water digestion as described by the Official Methods of Analysis (18, p. 524-525) has become somewhat of a standard method. This procedure was followed in general for the Pellet digestion.

Pass sample (usually in form of cossettes) through meat grinder fitted with plate having 1/4" perforations and mix

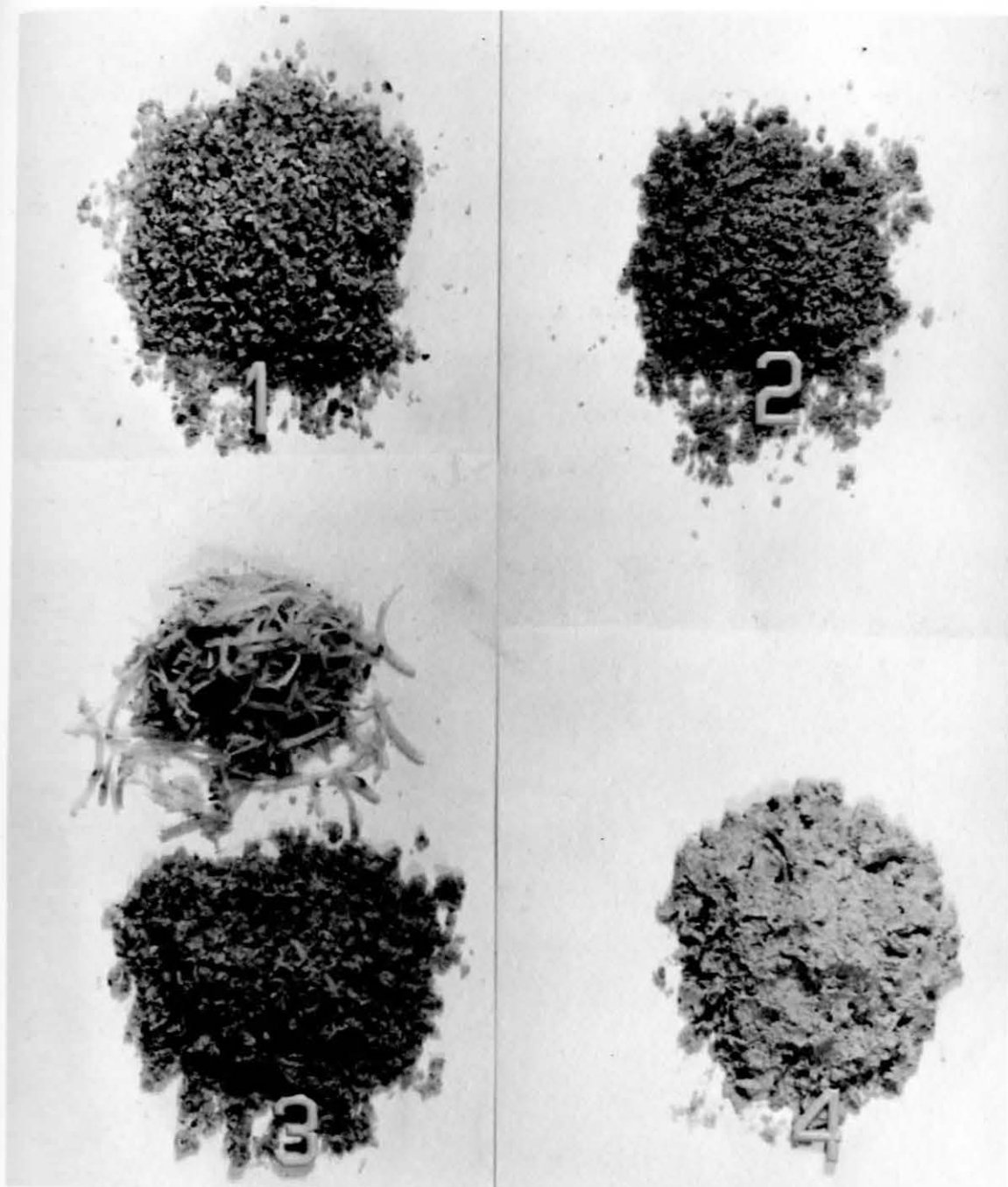


Figure 2. Pulp preparations. (1) Chopped in Hobart food cutter for 1 minute. (2) Chopped in Hobart food cutter for 10 minutes. (3) Cossettes above, after being ground in a meat grinder, below. (4) Rased pulp.

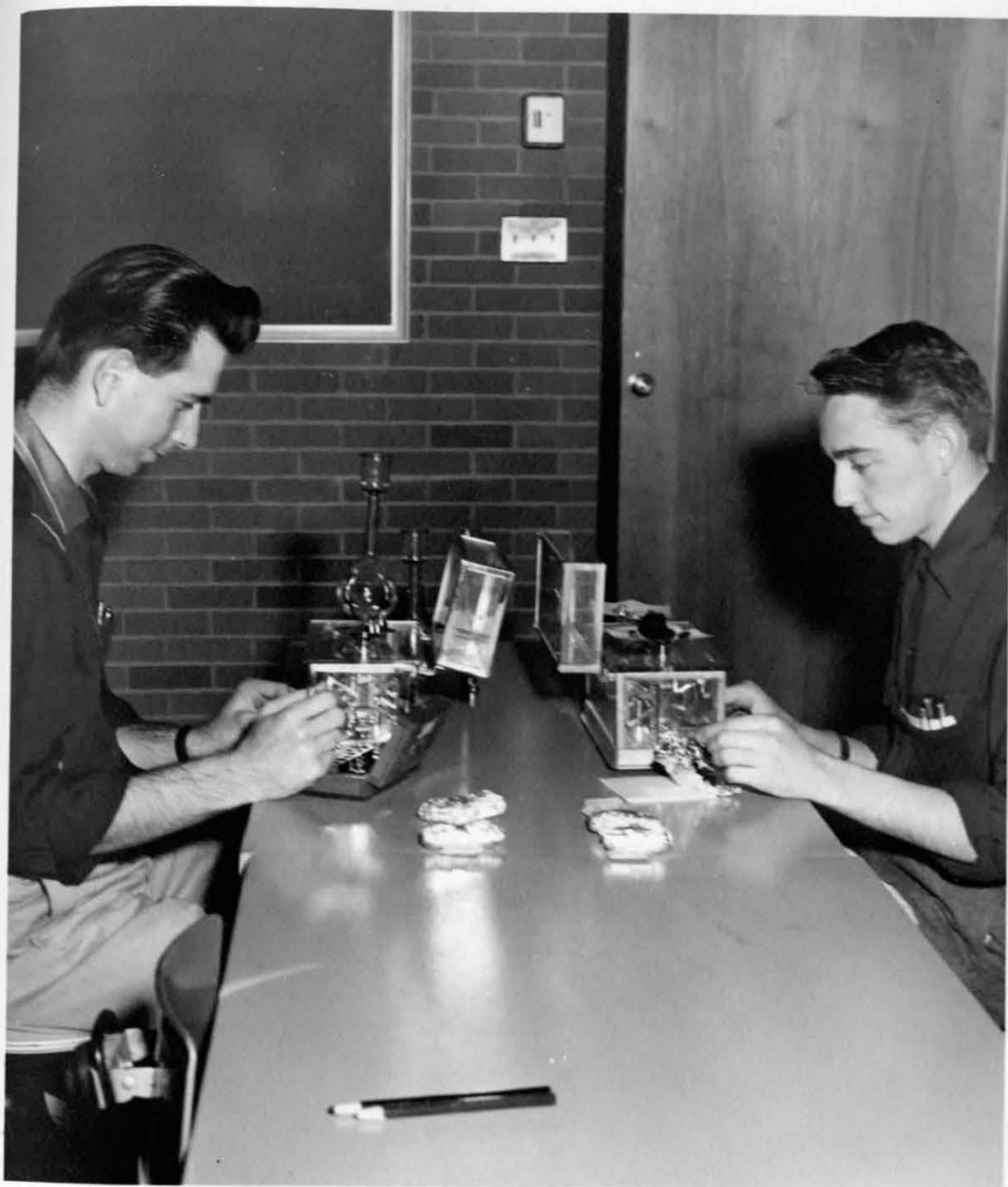


Figure 3. Methods of weighing samples. Weighing pulp into a Kohnrausch flask for the Pellet method (left). Weighing pulp onto tared onion skin paper for the Sachs-LeDocte method (right).

thoroughly. Weigh out 26 g of prepared sample and rinse into 201.0 ml. Kohlrausch flask, using ca 100 ml. of H₂O. Place flask under good vacuum 5-10 min. to remove air, carefully avoiding mechanical loss when vacuum is first applied. Add sufficient H₂O to make vol. of ca 175 ml. and digest in H₂O bath at 80°, supporting flask so that body is entirely immersed but is not in contact with heating element; 2 or 3 times during digestion period remove flask, mix contents by swirling, and after each agitation wash down pulp that adheres to walls of flask with little H₂O at 80°. After exactly 30 min. digestion fill flask to within 2-5 ml. of mark with H₂O at 80° and continue digestion exactly 10 min. longer. Cool to room temp. in H₂O bath. Add 6 ml. of basic Pb acetate soln. 29.18(a), and the small vol. of H₂O necessary to fill to mark. (Previous additions of H₂O and reagents should be so adjusted that not over 4 ml. of H₂O is required to make to vol.) Mix contents of flask well by shaking, allow to stand 5 min., shake again, and filter. Polarize in 400 mm. glass tube, after allowing solution to stand in immediate vicinity of saccharimeter at least 5 min. before reading. If vol. adjustment and polariscopic observation are made at 20°, reading gives per cent directly; if at other temps., apply formula in 29.19(a).

After the samples were weighed into the Kohlrausch flasks, the pulp was very carefully rinsed to the bottom by the use of glass stirring rods and squeeze-type wash bottles. Enough distilled water was added to make the total volume about 100 ml. and the flask was placed under a vacuum for 5 minutes or more. The volume was then brought to about 175 ml. with more water and the flask was placed in a constant temperature water bath. The time was then recorded to the nearest minute.

Four of the samples from C₁, C₂, C₃, and C₄ which had been weighed into the Kohlrausch flasks were placed in a water bath at 80° C. while the other 4 sets were placed in a water bath at 55° C. This made a total of 16 samples in each bath. Each batch of 4 samples in both baths was subjected to four different lengths of digestion time as outlined below. The initial digestion periods (T₁, T₂, T₃, and T₄) were 15 minutes, 30 minutes, 1 hour, and 2 hours. During these periods each flask was

removed from the bath briefly three times, and contents were mixed by swirling, and the adhering pulp was washed down by a small amount of hot water. The flasks were kept in sequence and order and were held above the bottom of the bath by the use of stiff wire netting or hardware cloth. Forced air was bubbled into the water baths to maintain constant temperature and circulation during digestion. Figure 4 shows the general laboratory setup for the digestion procedures. At the termination of the initial digestion time, the flask was removed, swirled, and enough hot water was added to bring it approximately to volume. It was then digested for another short period. The supplementary digestion time was 10 minutes for all flasks except T₁ which was left in the bath for 5 additional minutes. The flasks were then removed and cooled to 20° C. in a cold water bath. The small amount of water necessary was added to bring the contents to the 201.0 ml. mark. If any foam or air bubbles remained, a few drops of either were added before the solution was made to volume. The flask was then shaken well, and a small amount was filtered into a vial for total dry solids determinations. About 1.5 gms. of Horne's dry lead subacetate were added and the contents of the flask were well mixed by shaking. After allowing it to stand about 5 minutes, the flask was again shaken and the contents filtered as shown in figure 5.

Sachs-LeDocte method

While there are various modifications of the Sachs-LeDocte method, a typical procedure is as follows:

On a tared onion skin paper, 4 inches square, weigh rapidly 26 gms. of finely-divided, well-mixed beet pulp, and transfer, paper and all, to a monel metal capsule. Add from an automatic pipette 177 ml. of lead water



Figure 4. Digestion procedures. From left to right: 55° C. Pellet method; 55° C. Sachs-LeDocte method; 80° C. Pellet method; Sachs-LeDocte cold (20° C.) method. Swirling and rinsing for Pellet method shown on far left; 177 ml. automatic pipette for Sachs-LeDocte method shown on far right.



Figure 5. Clarification and filtering. Filtration for total dry solids determinations in background. Clarification and filtration for sucrose determinations in foreground.

(7° Brix, basic lead acetate solution). Cover with the iron ring and its rubber envelope, press down to seal, and shake vigorously for a few seconds. Filter the contents and polarize in a 400 mm. continuous tube (15, p. 95; 16). As a further precaution, after the first shaking, the capsule may be allowed to stand for 20 minutes or more, and again shaken. If the particles of pulp are not fine enough for cold-water digestion, the closed capsule, after being shaken, is digested in a water bath at about 80° C. for 30 minutes, then cooled to 20° C. It is again well shaken, the contents are filtered and polarized (15, p. 96). This procedure in general was used for the Sachs-LeDocte analysis in this experiment.

As the pulp samples were weighed out on the onion skin paper for the Sachs-LeDocte digestion, they were transferred, paper and all, to a heavy glass beaker of about 300 ml. volume. From an automatic pipette, 177 ml. of distilled water was added. The glass was sealed with an iron ring and rubber envelope cover and was shaken well to mix. The time was recorded to the nearest minute.

Four of the samples of C₁, C₂, C₃, and C₄ to be run by the Sachs-LeDocte method were set in a hot water bath at 55° C., while the other four were set aside at room temperature (20° C). (See figure 4.) The total digestion times of T₁, T₂, T₃, and T₄ were 15 minutes, 30 minutes, 1 hour, and 2 hours, respectively. After digestion had proceeded in the T₁, T₂, T₃, and T₄ beakers for 5, 10, 15, and 20 minutes, respectively, the beakers were shaken and a small sample was removed from each and filtered for total dry solids determination. About 1.5 grams of Horne's dry lead subacetate were added and the contents were mixed by shaking. Digestion was then continued for the remainder of the total period, at which time the beakers

were shaken and the contents filtered.

The room temperature was thermostatically controlled at 20° C. to insure the proper temperature of all instruments, equipment, distilled water and samples.

Identity of each sample was maintained by its specific location in the water baths and by two small cards with systematic coding of sample identification which were kept with the sample from weighing to filtering. One of these cards was placed with the filtered sample for total dry solids analysis while the other one accompanied the clarified solution for sucrose determination. The identity of each sample, along with the initial time that digestion was begun, the time to be brought to volume (Pellet method) or the time to have a sample taken for total dry solids determination (Sachs-LeDocte method), and the time when the digestion period should be terminated were recorded on the blackboard so that the progress of each sample could be easily watched and controlled. The general laboratory procedures may be seen in figure 6.

Figure 7 shows the temperature-controlled Bausch and Lomb precision sugar refractometer #33-45-01 which was used to determine total dry solids. Per cent sucrose was obtained directly by polarizing the clarified solutions in a 400 mm. continuous tubed Bausch and Lomb saccharimeter, #33-62-05 having a Jellet single prism polarizer with a fixed half-shade angle of approximately 7° as shown in figure 8.

Related studies

Other related studies were carried out which would add value and interpretation to this research.



Figure 6. General laboratory procedures.

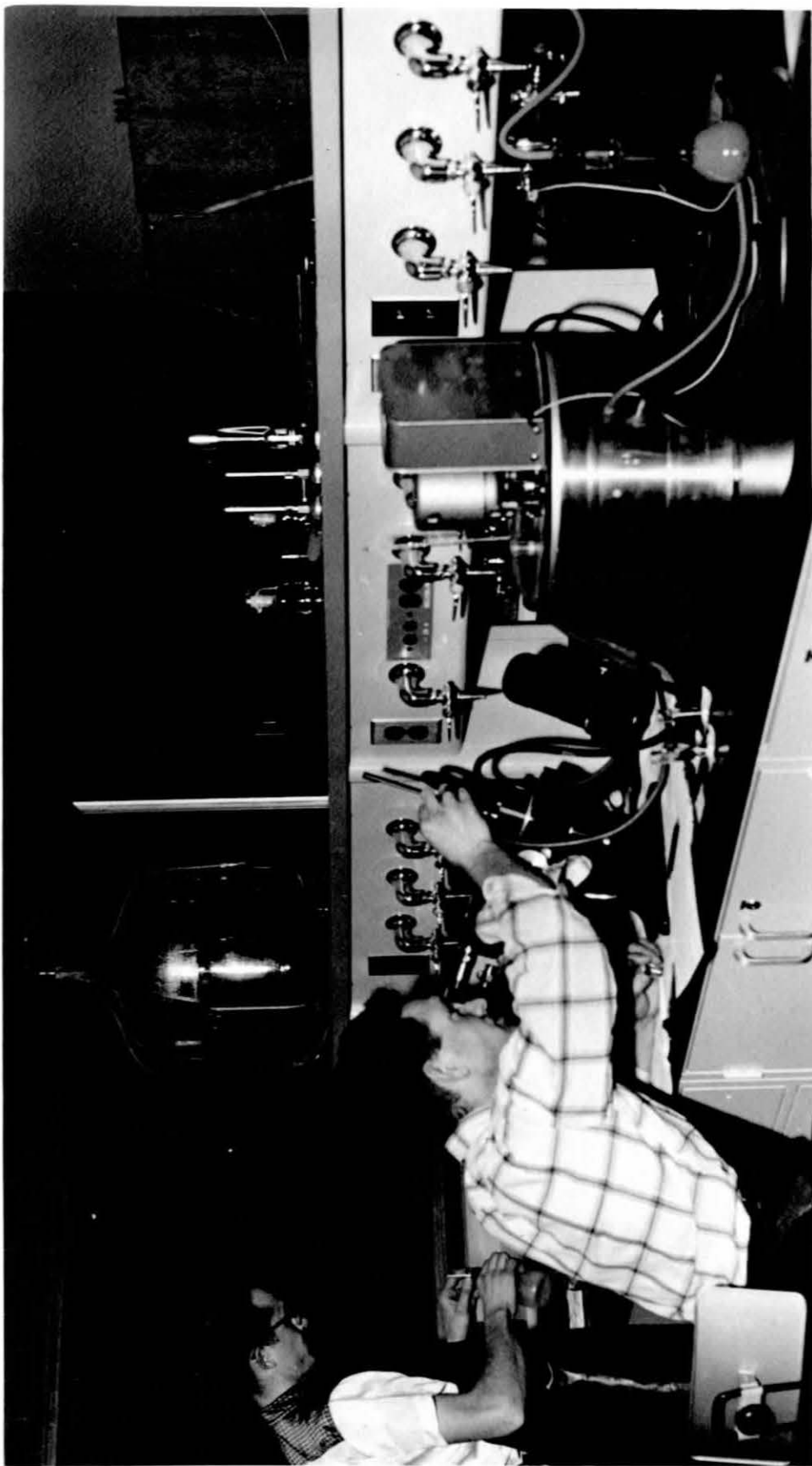


Figure 7. Bausch and Lomb precision sugar refractometer #33-45-01.



Figure 8. Bausch and Lomb saccharimeter #33-62-05.

Sacharimetry versus copper reduction. A separate experiment was run to compare a standard hot water digestion plus simple polarization method with an alcohol extraction plus copper reduction method of sucrose analysis. Duplicate determinations were made on 50 beets by both methods. In the digestion plus polarization method the same procedure was followed as described on pages 24 and 25, except that dry basic lead acetate was added after bringing to volume in order that purity determinations could also be made. The copper reduction method of analysis as described in the Official Methods of Analysis (18, pp. 347, 348, 507, 508) was employed.

Coarseness of sample. A study was made to determine the relative size or coarseness of the beet pulp samples as prepared by the four methods. This was done by arranging a nest of sieves of the following mesh: 4, 8, 10, 20, 40, 60, and 140 (number represents number of openings per linear inch) in sequence from coarse on top to fine at the bottom. One hundred grams of beet pulp were placed in the top sieve; the lid was put on and the entire nest was immersed in water. They were kept in continual agitation and rotation for about 30 minutes, or until no further separation seemed to occur. This was repeated with 100 grams of each of the other three sample preparations. The entire procedure was then repeated the next day on fresh sample preparations except that 150 grams of sample were used. After each test, all the sample that remained in each sieve was carefully removed, placed in metal containers, labeled, and put in a drying oven. After about 48 hours, or when completely dry, they were removed and weighed. The per cent dry pulp of total dry weight obtained from each sieve was determined.

Sampling error due to quartering of beets. Since one of the basic

assumptions in the sampling procedure of this research, as well as nearly all present sampling techniques in the analysis of sugar beets, is that any quarter or wedge-shaped segment of a beet, taken in line with its axis, is equal in concentration of all constituents to a similar segment in any other portion of that beet, it would be well to test the reliability of this theory.

An experiment was carried out to test the sucrose content and percent purity variations obtained due to sampling quarters of beets both when beets are bulked and when sampled individually. Thirty beets from one nitrogen level were washed thoroughly and all 30 beets were cut into four equal quarters through the center. One quarter from each beet was picked at random and bulked in four equal lots. Each lot was processed separately but identically by chopping for 3 minutes in the Hobart food cutter. Each batch was mixed thoroughly, and approximately 30 gram samples were quickly weighed out, labeled and placed in dry ice. When enough samples from the four batches of bulked quarters from all three nitrogen levels were prepared, they were taken to the locker and frozen. Four quarters from 10 beets were also prepared in a similar manner but each quarter was chopped for 3 minutes, labeled separately and frozen for future analysis.

Duplicate sucrose and purity determinations were made on all of the bulked samples, and single determinations were made on all quarters of the 10 single beets, following the standard digestion procedure described on pages 24 and 25. Duplicates were later run on all quarters of the individual beets which seemed to vary from the other quarters of the same beet.

The laboratory analyses were begun on December 28, 1956, and were completed on March 6, 1957. The beets were still in generally good condition on March 9 after all analyses were completed, as shown in figure 1.

RESULTS AND DISCUSSION

Sucrose content

Sucrose content data were obtained from the 1152 sugar beet samples of the main study and the data subjected to statistical analysis. The results are given in table 1. The analysis of variance shows a significant difference due to all of the main effects as well as all but three of the two-way interactions. Most of the differences are highly significant.

All factors or interactions which showed a significant difference were plotted in graph form and a test was made to determine significant differences between levels or treatments by multiplying the standard error of the treatment means by t at $t_{.05}$ and $t_{.01}$.

Replications 1 and 2 are significantly different from replication 3. One would expect a gradual decrease in sucrose content of the sugar beets due to time of storage. This was the case, as shown in figure 9.

Freezing the samples before analysis gave a significant increase in sucrose per cent, as shown in figure 10. This might be expected since other treatments used in this study apparently have less effect when the cell membranes of the plant material are disrupted by freezing.

It was found that the sucrose content decreases as nitrogen level increases, in agreement with reports by Haddock (11), Wooley (26), and others. Figure 11 shows that N_0 and N_1 are significantly higher in sucrose content than N_2 .

There is a highly significant difference between P_1 and P_2 , P_3 , and P_4 as shown in figure 12. P_4 is significantly different from P_2 . One

Table 1. Analysis of variance for sucrose content of sugar beets

Source of variation	Degrees of freedom	Sums of squares	Mean square	F	.05	.01
Replications	2	144.3773	72.1887*	19.45	19.00	99.00
Freezing	1	156.5712	156.5712*	42.19	18.51	98.49
Error (a)	2	7.4229	3.7115			
Nitrogen	2	365.5640	182.7820**	12.65	4.46	8.65
Freez. x Nit.	2	2.8306	1.4153	0.10	4.46	8.65
Error (b)	8	115.5759	14.4470			
Process	3	426.1792	142.0597**	94.73	2.83	4.29
Proc. x Freez.	3	219.2584	73.0861**	48.74	2.83	4.29
Proc. x Nit.	6	11.8244	1.9707	1.31	2.32	3.26
Error (c)	42	62.9839	1.4996			
Coarseness	3	330.3053	110.1018**	327.68	2.61	3.80
Coars. x Proc.	9	288.0485	32.0054**	95.25	1.89	2.43
Coars. x Freez.	3	132.3265	44.1088**	131.28	2.61	3.80
Coars. x Nit.	6	4.8294	0.8049*	2.40	2.10	2.82
Time	3	56.4270	18.8090**	55.98	2.61	3.80
Time x Coars.	9	21.2162	2.3574**	7.02	1.89	2.43
Time x Proc.	9	46.6071	5.1786**	15.41	1.89	2.43
Time x Freez.	3	13.1745	4.3915**	13.07	2.61	3.80
Time x Nit.	6	1.5531	0.2589	0.77	2.10	2.82
Error (d)	1029	345.7464	0.3360			
Total	1151	2752.8218				

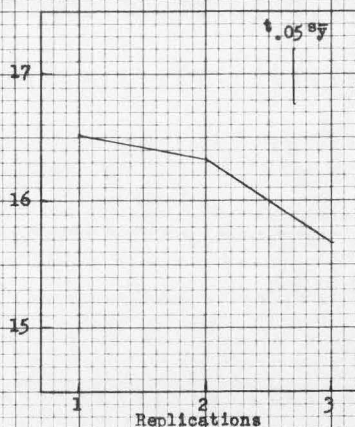


Figure 9. Influence of replications (or storage) on the sucrose content of sugar beets.

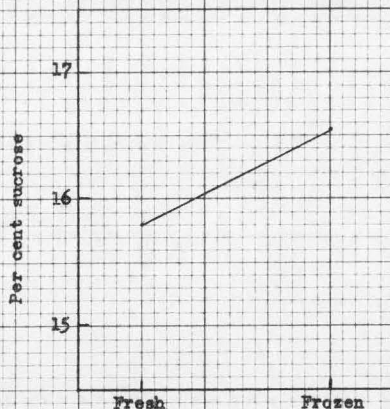


Figure 10. Influence of freezing samples on the extraction of sucrose from sugar beets.

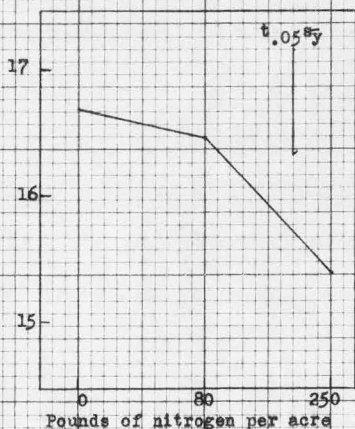


Figure 11. Influence of nitrogen fertilization on the sucrose content of sugar beets.

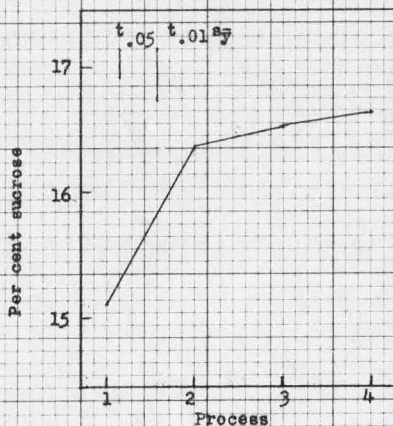


Figure 12. Influence of process on the extraction of sucrose from sugar beets.

would expect this result because of the inefficiency of the cold digestion in extracting sucrose from coarse beet pulp. Between all degrees of coarseness there are highly significant differences except between C_2 and C_3 as shown in figure 14. However, they also are significantly different at the .05 level. This would be expected because of the semi-permeability of intact cell membranes and the difficulty of sucrose passing through by diffusion.

Length of digestion periods show a continual increase in sucrose extraction from 15 minutes to 2 hours in figure 17. Highly significant differences occur between all the time periods except between T_3 and T_4 , where no significant change in sucrose content occurs. This is as expected because diffusion of sucrose is very rapid during the first part of the digestion period and will gradually level off as sucrose concentration comes to equilibrium both inside the pulp cells and in the solution outside.

A few general observations and statements regarding significant differences due to interactions will help in understanding the causes of differences and in deciding which methods of analysis are of value.

Figure 13 shows that while sucrose analysis using frozen samples is not nearly so greatly influenced by process as the unfrozen pulp, it is evident that only P_3 and P_4 give about the same average sucrose content in all samples. One factor that may contribute substantially to the high sucrose per cent as obtained by P_2 with several of the interactions is that a small sample (about 10 ml.) was taken soon after digestion to obtain a total dry solids determination as explained previously. It became evident that this is not long enough as sucrose and other constituents

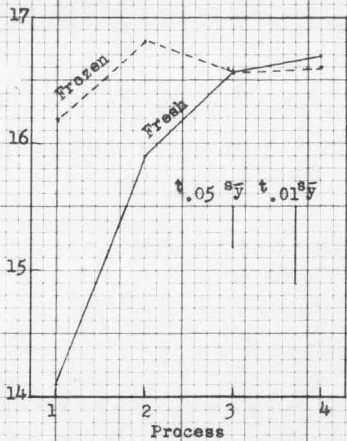


Figure 13. Influence of process and freezing on the extraction of sucrose from sugar beets.

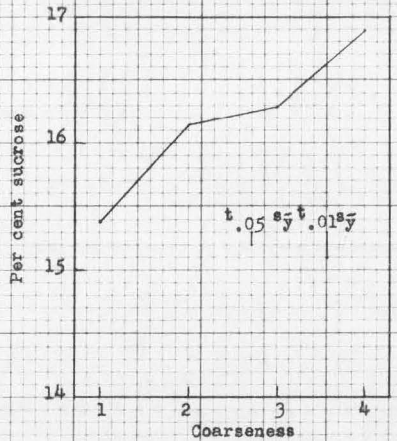


Figure 14. Influence of coarseness on the extraction of sucrose from sugar beets.

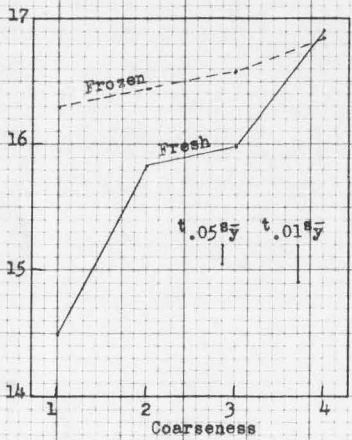


Figure 15. Influence of coarseness and freezing on the extraction of sucrose from sugar beets.

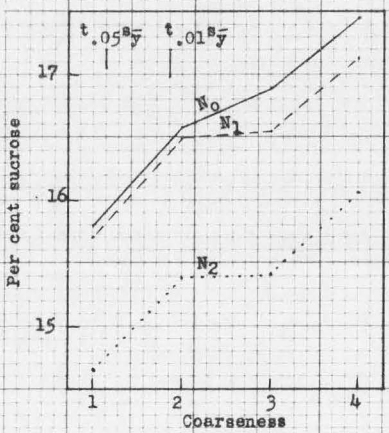


Figure 16. Influence of coarseness and nitrogen fertilization on the extraction of sucrose from sugar beets.

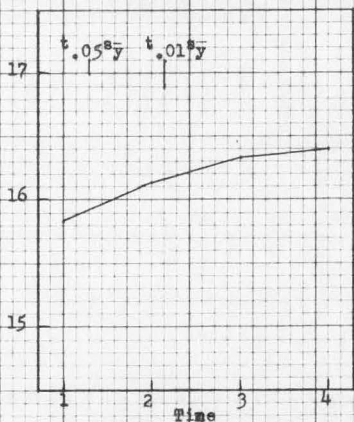


Figure 17. Influence of time on the extraction of sucrose from sugar beets.

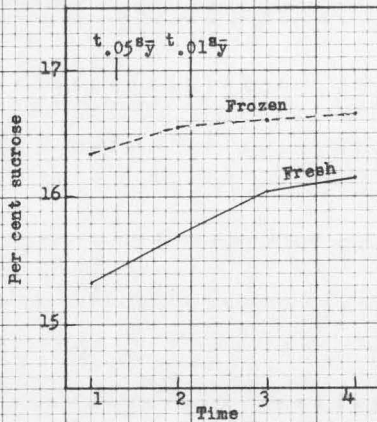


Figure 18. Influence of time and freezing on the extraction of sucrose from sugar beets.

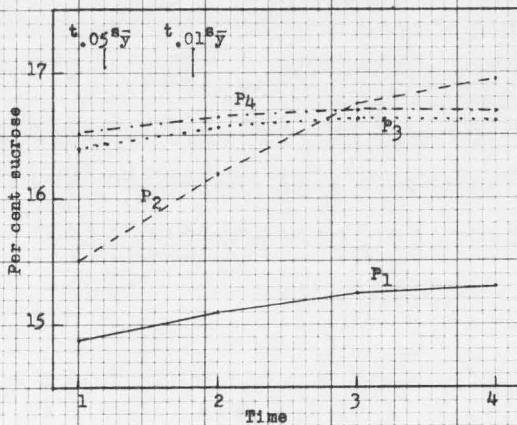


Figure 19. Influence of time and process on the extraction of sucrose from sugar beets.

are still being extracted. Since considerable sucrose was extracted after this sample was taken for total dry solids determination, the sucrose percent in the remaining solution was higher than the true value. It is also quite possible that the pulp was not mixed adequately before the sample was taken. Furthermore, it is apparent that the pipette volume for the Sachs-LeDocte process should be larger than 177 ml. in agreement with Bachler (1).

The interaction between process and coarseness as shown in figure 21 again gives evidence of the greater efficiency of processes 3 and 4 in extracting sucrose from all degrees of coarseness of pulp samples. P_1 is of no apparent value in sucrose analysis except with pulp of extreme fineness. P_2 is also too variable throughout the four levels of coarseness.

All interactions between degree of coarseness and fresh versus frozen pulp are significant except C_4 , as seen in figure 15.

Figure 16 shows significant differences over all degrees of coarseness and levels of nitrogen except between N_0 and N_1 at C_1 and C_2 .

There are no significant differences due to time with rasped pulp as shown in figure 20. There are no significant differences between C_2 and C_3 at any time except T_1 . T_3 is not significantly different from T_4 at any given coarseness except C_1 where it is barely significant at the .05 level.

Figure 19 indicates that only P_3 and P_4 are not greatly affected by time; there are no significant differences between them at any time. However, there are significant differences between T_1 and T_3 over all processes, and between T_1 and T_2 over all processes except P_4 .

Frozen samples give significantly higher sucrose analysis over all time intervals than unfrozen samples, as seen in figure 18. Unfrozen pulp

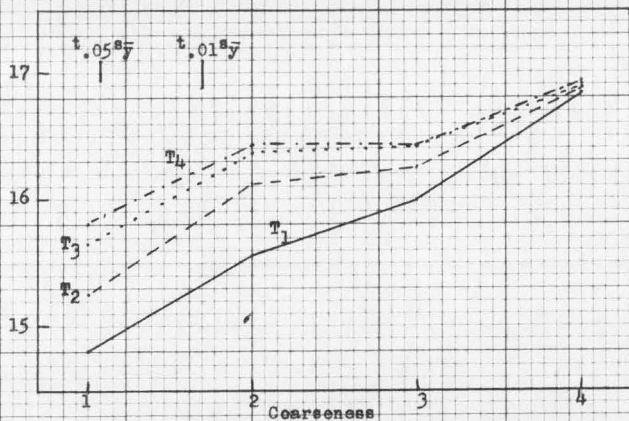


Figure 20. Influence of coarseness and time on the extraction of sucrose from sugar beets.

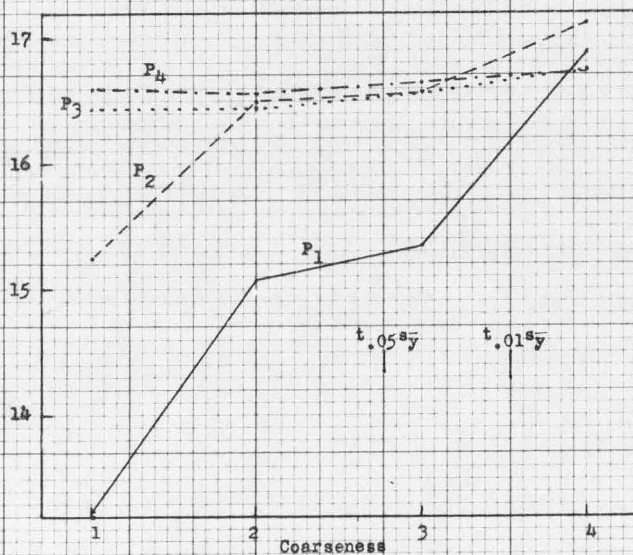


Figure 21. Influence of coarseness and process on the extraction of sucrose from sugar beets.

shows significant increases in sucrose percentage with each increase in time interval except between T_3 and T_4 . Frozen pulp digestion is not greatly affected by time but T_1 is significantly different from T_2 , T_3 , and T_4 .

Per cent purity

The analysis of variance for the per cent purity data is shown in table 2. The results indicate that of the main effects, only process and coarseness give highly significant differences. Five two-way interactions show significant overall differences and tests were made to determine significant differences between treatments as was done for the sucrose data.

Figure 22 shows that P_1 and P_2 are significantly different from P_3 and P_4 . It is important to note that the over-all data for P_1 and P_2 are quite unrealistic, showing purity percentages greater than 100 per cent. This is due to the procedure used in taking a sample for total dry solids determinations for P_1 and P_2 .

Since these samples were obtained after only a portion of the total digestion period had elapsed, while sucrose and probably other constituents of the beet juices continued to be digested out of the pulp, the purity percentages calculated from these data would be too high. Consequently, the purity data as determined on all but the rasped samples from P_1 and P_2 are of little meaning and value. It is also of interest to note that P_2 is significantly higher in purity per cent than P_1 . This may be explained by the fact that the ratio of sucrose extracted after total dry solids sample was taken to total dry solids extracted before was greater with P_2 where heat was applied than with P_1 . This is even more apparent in figure 23 which shows the results of the process by freezing interaction.

Table 2. Analysis of variance for per cent purity of sugar beets

Source of variation	Degrees of freedom	Sums of squares	Mean square	F	.05	.01
Replications	2	205.4702	102.7351	0.36	19.00	99.00
Freezing	1	34.4796	34.4796	0.12	18.51	98.49
Error (a)	2	576.9836	288.4918			
Nitrogen	2	1093.3026	546.6513	3.81	4.46	8.65
Freez. x Nit.	2	498.8940	249.4470	1.74	4.46	8.65
Error (b)	8	1147.9330	143.4916			
Process	3	35264.1253	11754.7084**	253.88	2.83	4.29
Proc. x Freez.	3	1243.0878	414.3626**	8.95	2.83	4.29
Proc. x Nit.	6	480.0602	80.0100	1.73	2.32	3.26
Error (c)	42	1944.6184	46.3004			
Coarseness	3	31370.4369	10456.8123**	416.47	2.61	3.80
Coars. x Proc.	9	20826.4384	2314.0487**	92.16	1.89	2.43
Coars. x Freez.	3	525.3828	175.1276**	6.97	2.61	3.80
Coars. x Nit.	6	421.3499	70.2250*	2.80	2.10	2.82
Time	3	88.9571	29.6524	1.18	2.61	3.80
Time x Coars.	9	179.2961	19.9218	0.79	1.89	2.43
Time x Proc.	9	266.0171	29.5575	1.18	1.89	2.43
Time x Freez.	3	15.3476	5.1159	0.20	2.61	3.80
Time x Nit.	6	460.1614	76.6936**	3.05	2.10	2.82
Error (d)	1029	25836.2792	25.1081			
Total	1151	122478.6212				

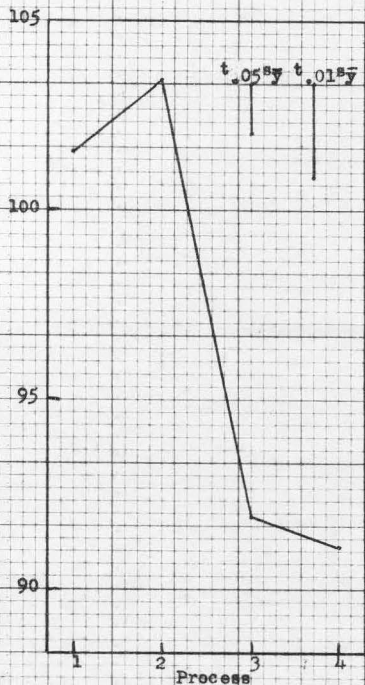


Figure 22. Influence of process on purity analysis of sugar beets.

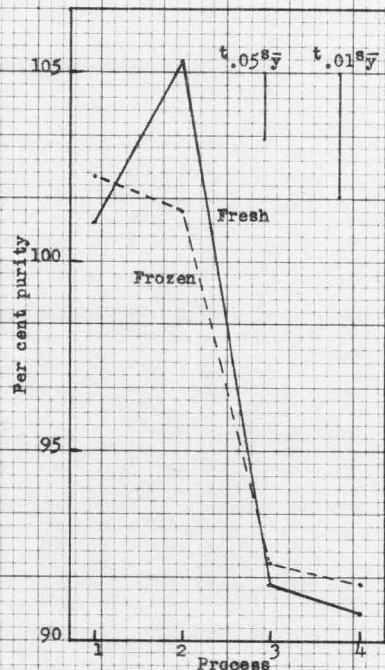


Figure 23. Influence of process and freezing on purity analysis of sugar beets.

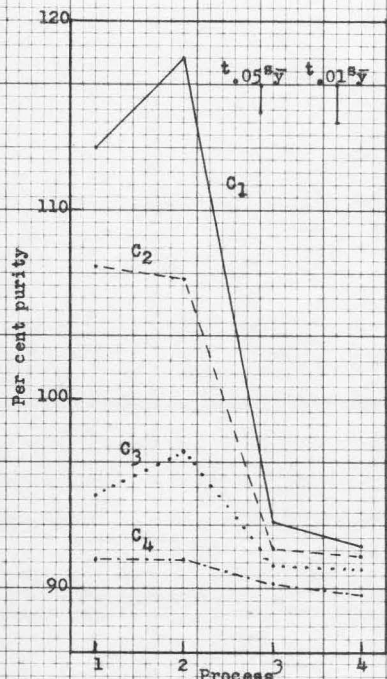


Figure 24. Influence of process and coarseness on purity analysis of sugar beets.

Temperature has a minor effect over the digestion period with frozen samples but extraction of sucrose from unfrozen pulp is greatly influenced by temperature over the digestion period. Figure 24 shows that C_4 is not so greatly affected by process although P_1 and P_2 are significantly higher than P_4 . There are no significant differences between P_3 and P_4 for any degree of coarseness. However, C_1 and C_2 are significantly higher than C_4 for all processes. C_1 is significantly higher than C_3 for all processes except P_4 .

Per cent purity is greatly affected by coarseness of sample as seen by figure 27. All degrees of coarseness give highly significant differences. The interactions of freezing with coarseness and of nitrogen levels with coarseness also show some significant differences as seen in figures 25 and 26. However, the results are somewhat confounded with the unrealistic data from P_1 and P_2 so it is difficult to draw definite and sound conclusions from these differences. The same is true with the time by nitrogen interactions from figure 28. It is of importance that in all cases N_2 is lower in purity percentage than N_0 and N_1 as would be expected (11). N_1 is significantly higher than N_0 at C_1 (figure 26) and at T_1 (figure 28).

Related studies

Saccharimetry versus copper reduction. The data from the experiment comparing an aqueous digestion--saccharimetry method--to an alcohol-extraction and copper-reduction method of sucrose analysis are presented in table 3. The sucrose analysis by saccharimetry gives a definite and consistent increase over the copper reduction procedure. The only exception to this is from beet number 38 which gives a slightly lower sucrose per

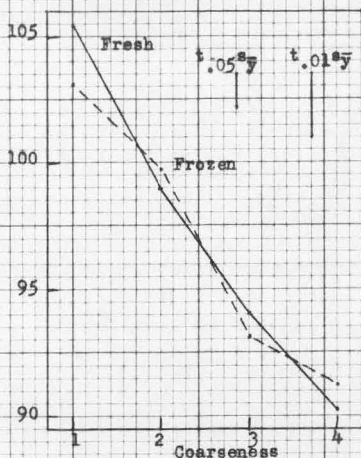


Figure 25. Influence of coarseness and freezing on purity analysis of sugar beets.

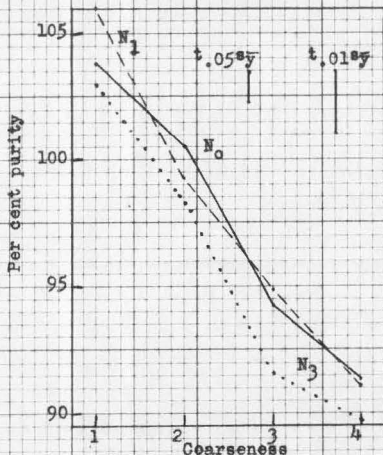


Figure 26. Influence of coarseness and nitrogen fertilization on purity analysis of sugar beets.

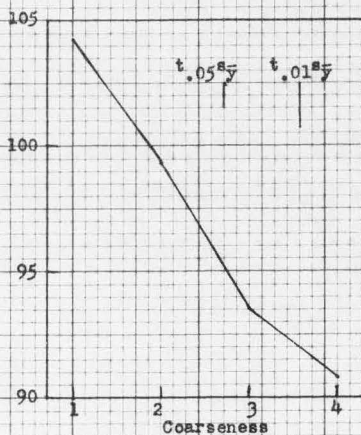


Figure 27. Influence of coarseness on purity analysis of sugar beets.

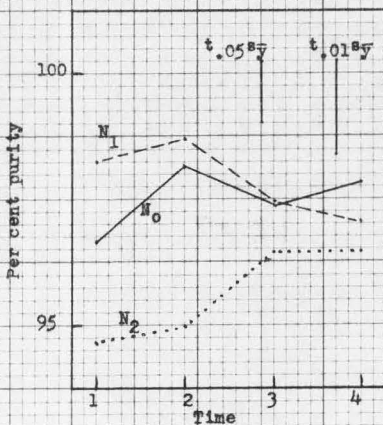


Figure 28. Influence of time and nitrogen fertilization on purity analysis of sugar beets.

Table 3. Comparison study of saccharimetry versus copper reduction for sucrose analysis of sugar beet pulp

Sample no.	Average sucrose % by sacc.	Average sucrose % by Cu red.	% sucrose diff. sacc.-Cu red.	Sample no.	Average sucrose % by sacc.	Average sucrose % by Cu red.	% sucrose diff. sacc.-Cu red.
1	17.47	15.80	1.67	26	14.87	14.10	0.77
2	16.40	15.55	0.85	27	15.20	14.60	0.60
3	17.03	16.05	0.98	28	17.57	17.35	0.22
4	15.67	15.35	0.32	29	15.60	14.85	0.75
5	14.70	14.10	0.60	30	15.77	15.30	0.47
6	15.70	15.55	0.15	31	15.93	15.45	0.48
7	17.27	17.05	0.22	32	16.97	16.65	0.22
8	16.10	15.70	0.40	33	17.10	16.75	0.35
9	15.40	15.20	0.20	34	16.30	15.65	0.65
10	16.93	16.25	0.68	35	16.67	15.75	0.92
11	15.33	14.25	1.08	36	14.13	13.70	0.43
12	16.10	15.60	0.50	37	14.77	13.80	0.97
13	15.47	14.75	0.72	38	15.80	15.90	-0.10
14	17.47	17.00	0.47	39	14.67	14.20	0.47
15	16.70	16.15	0.55	40	10.83	10.30	0.53
16	14.80	13.90	0.90	41	13.53	13.35	0.18
17	16.83	16.40	0.43	42	14.93	14.45	0.48
18	13.60	12.95	0.65	43	15.57	14.90	0.67
19	13.87	13.10	0.77	44	14.90	14.45	0.45
20	15.87	15.15	0.72	45	16.30	15.90	0.40
21	15.13	14.25	0.88	46	15.10	14.50	0.60
22	16.20	15.25	0.95	47	14.27	13.70	0.57
23	16.60	15.95	0.65	48	16.07	15.50	0.57
24	14.77	14.20	0.57	49	16.30	15.75	0.55
25	15.50	14.35	1.15	50	16.13	15.50	0.63

cent of an average of two determinations by saccharimetry. Since these two analyses on the same beet are so far apart (16.1 per cent and 15.5 per cent sucrose) we could suspect that there may have been some error such as in weighing the sample, mixing the pulp, etc. The differences between the two methods in per cent sucrose range from -0.10 per cent on beet #38 to 1.67 per cent on beet #1, with an over-all average increase for the saccharimetry method of 0.60 per cent.

The lack of agreement between these two basically different but widely accepted procedures ^{is} ~~are~~ apparently much greater than commonly supposed. By far the greatest proportion of the literature on the subject emphasizes the seemingly minor and relatively unimportant considerations and differences within only one method or between similar methods of sucrose analysis, such as the precise volume of solution to add or the volume of flask to use, whether volume or marc hydrate, marc anhydrate, or marc hydrate plus lead precipitate should be used for volume correction. Even if the assumed marc volume was 1 ml. greater or less than the true marc volume, the difference in the polarization of a beet containing 16 per cent sucrose would be only 0.08 per cent sucrose. It is evident that the factor or factors which are responsible for the difference between the two methods are quite consistent between samples from the same beet, but vary appreciably between samples from different beets. The average deviation between samples of the same beet taken over all 50 beets is 0.08 per cent sucrose for the copper reduction method and 0.09 per cent sucrose for the saccharimetry method. Therefore, the precision or consistency of both methods is sufficient to assume that the cause of the increase in sucrose per cent by saccharimetry is not due to chance and is

not a constant factor inherent in the procedures, but varies greatly between beets. This would indicate that at least part of the increase in sucrose per cent by simple polarization may be due to other optically active substances having predominantly dextrorotatory action either naturally or induced by the effects of lead subacetate on their optical activity.

These 50 beets are also being quantitatively analyzed for various other constituents such as raffinose, glutamate, total anionic constituents, galactanol, malic acid, oxalic acid, and amino nitrogen. A multiple correlation will be run to determine if the amount of increase in sucrose content by saccharimetry over copper reduction determinations ¹⁵are in some way correlated with the relative concentration of these various constituents.

Coarseness of sample. The data from the study to determine relative coarseness of each sample preparation are given in table 4.

Sampling error due to quartering of beets. The results of the experiment to test the assumption that any quarter or wedge-shaped segment of a beet taken in line with its axis is a true and representative sample of the entire beet are shown in tables 5 and 6. The consistency of sucrose determinations where duplications were made and also between quarters is striking evidence of the precision obtainable by this method when extreme care is taken to ensure thorough mixing and accurate weighing of samples. It is also evident that when a sufficient number of carefully-cut quarters are bulked and processed properly, they do represent a true sample of the entire lot of beets.

However, the data obtained from analysis of individual quarters from single beets show that we are not correct in our basic assumption when it

Table 4. Determination of relative size of sugar beet pulp particles prepared by the four methods

Method of preparation	Sieve no.	Area of each opening (sq.mm.)	Grams dry wt. per 100g wet wt.	Grams dry wt. per 150g wet wt.	Average	% dry wt. of total dry wt.	% dry wt. of total dry wt.	Av. % dry wt. of total dry wt.
Chopped for 1 min.	4	22.66	0.68	0.85		4.4	3.9	4.2
	8	5.66	9.48	14.83		61.0	67.5	64.2
	10	4.00	1.76	1.95		11.4	8.9	10.1
	20	.71	3.33	4.08		21.4	18.6	20.0
	40	.18	0.23	0.19		1.5	0.9	1.2
	60	.06	0.05	0.04		0.3	0.2	0.3
Total dry wt.			15.53	21.94				
% total dry wt. of total wet wt.			15.53	14.63	15.08			
Cossetted and ground	4	22.66	0.50	0.22		5.2	2.0	3.6
	8	5.66	5.52	7.62		57.2	67.2	62.2
	10	4.00	0.81	0.74		8.4	6.5	7.4
	20	.71	2.36	2.29		24.4	20.2	22.3
	40	.18	0.37	0.43		3.8	3.8	3.8
	60	.06	0.09	0.03		1.0	0.3	0.7
Total dry wt.			9.65	11.33				
% total dry wt. of total wet wt.			9.65	7.55	8.60			
Chopped for 10 min.	8	5.66	0.04	0.11		0.4	0.8	0.6
	10	4.00	0.11	0.27		1.2	2.0	1.6
	20	.71	8.08	11.83		85.2	88.2	86.7
	40	.18	0.96	0.88		10.1	6.5	8.3
	60	.06	0.21	0.26		2.2	2.0	2.1
	140	.01	0.08	0.06		0.9	0.5	0.7
Total dry wt.			9.48	13.41				
% total dry wt. of total wet wt.			9.48	8.94	9.21			
Rasped	4	22.66	0.50	0.28		9.7	3.8	6.8
	8	5.66	0.16	0.40		3.1	5.4	4.2
	10	4.00	0.18	0.30		3.5	4.0	3.7
	20	.71	2.00	3.50		38.6	47.3	42.9
	40	.18	1.26	1.89		24.4	25.5	25.0
	60	.06	0.75	0.66		14.5	8.9	11.7
	140	.01	0.32	0.38		6.2	5.1	5.7
Total dry wt.			5.17	7.41				
% total dry wt. of total wet wt.			5.17	4.94	5.06			

Table 5. Sucrose content variations from quartering beets when the quarters from 30 beets are bulked into four equal lots (one quarter per beet per lot)

Nitrogen level of beets	Bulked quarters lot no.	Dup.	Per cent total dry solids	Purity per cent	Per cent sucrose	
0 pounds per acre	1	a	17.53	90.7	15.9	
		b	18.10	87.3	15.8	
	2	a	17.68	89.9	15.9	
		b	18.10	87.3	15.8	
	3	a	17.68	89.9	15.9	
		b	18.38	86.0	15.8	
	4	a	17.68	89.9	15.9	
		b	18.24	87.2	15.9	
80 pounds per acre	1	a	17.68	87.7	15.5	
		b	18.24	85.0	15.5	
	2	a	18.10	86.7	15.7	
		b	18.10	85.1	15.4	
	3	a	17.25	88.7	15.3	
		b	17.96	85.7	15.4	
	4	a	17.82	86.4	15.4	
		b	17.96	85.7	15.4	
	250 pounds per acre	1	a	17.25	85.2	14.7
			b	17.25	85.8	14.8
		2	a	17.25	85.2	14.7
			b	17.11	85.3	14.6
3		a	16.68	88.7	14.8	
		b	16.96	87.3	14.8	
4		a	16.96	86.7	14.7	
		b	17.25	85.8	14.8	

Table 6. Sucrose content variations from individual quarters from individual beets

Beet no.	Quarter no.	Dup. no.	Per cent total dry solids	Per cent purity	Per cent sucrose	
1	1	a	16.11	85.7	13.8	
		a	16.11	85.7	13.8	
	2	a	16.25	84.9	13.8	
		a	16.25	87.4	14.2	
		b	16.25	87.4	14.2	
2	1	a	15.82	82.8	13.1	
		b	15.67	84.2	13.2	
	2	a	15.53	82.4	12.8	
		a	15.39	83.2	12.8	
		a	15.53	83.1	12.9	
3	1	a	16.53	87.7	14.5	
		a	16.68	86.9	14.5	
	3	a	16.96	87.3	14.8	
		b	16.11	91.2	14.7	
	4	a	17.11	85.9	14.7	
b	16.53	88.9	14.7			
4	1	a	17.11	90.6	15.5	
		b	17.53	87.8	15.4	
	2	a	17.53	90.1	15.8	
		a	17.53	90.1	15.8	
		a	17.68	89.4	15.8	
5	1	a	17.53	86.7	15.2	
		b	17.53	86.7	15.2	
	2	a	17.25	87.0	15.0	
		3	a	16.96	87.3	14.8
			b	16.68	88.1	14.7

Table 6. (continued)

Beet no.	Quarter no.	Dup. no.	Per cent total dry solids	Per cent purity	Per cent sucrose	
5	4	a	17.25	87.0	15.0	
6	1	a	18.38	89.2	16.4	
		a	18.53	89.0	16.5	
	b	18.53	89.0	16.5		
	3	a	18.38	89.2	16.4	
		a	18.24	89.9	16.4	
7	1	a	16.39	83.0	13.6	
		b	16.68	81.5	13.6	
	2	a	16.82	81.5	13.7	
		a	16.82	82.0	13.8	
	4	a	16.82	82.6	13.9	
		b	16.53	84.7	14.0	
8	1	a	17.82	88.7	15.8	
		b	17.25	91.0	15.7	
	2	a	18.68	86.7	16.2	
		a	18.38	89.2	16.4	
	3	b	18.68	87.8	16.4	
		a	17.82	89.8	16.0	
	9	1	a	18.53	92.8	17.2
b			17.96	95.8	17.2	
2		a	19.26	92.4	17.8	
		b	19.26	92.9	17.9	
3		a	18.68	92.6	17.3	
		a	18.97	92.8	17.6	
10		1	a	17.53	88.4	15.5
		2	a	17.68	88.2	15.6
	3	a	17.82	88.1	15.7	
	4	a	17.68	89.4	15.8	
		b	18.10	87.8	15.9	

concerns a single beet or a small lot of beets. Since this study made use of one-fourth of the beet in determining its accuracy in representing the entire beet, it would be reasonable to assume that a smaller segment such as is obtained with the rasp (which takes less than one-tenth of the total beet) would be subject to even more error and deviation from the true average composition of the beet, unless a considerable number of beets were to be sampled. However, bulked samples obtained from the rasp or meat grinder would not correct any inherent additive errors.

These data would suggest that it might be advantageous to continue the study to determine if there are correlations between the position of the beet in the field regarding direction of sunlight, irrigation water, fertilizer applications, etc., and variations in its content of sucrose and other constituents.

SUMMARY AND CONCLUSIONS

A split plot laboratory experiment was conducted to study the influence of nitrogen fertilization, freezing of pulp samples, and temperature of bath, the Sachs-LeDocte method versus the Pellet method, the coarseness of samples, and the length of digestion time on sucrose and purity analysis by aqueous digestion and saccharimetry.

There were highly significant differences in sucrose per cent due to all of the treatment main effects except freezing, which was significant at the .05 level.

The effect of freezing shows that sucrose is more efficiently extracted from the beet pulp that has been previously frozen.

The main effects of nitrogen fertilization show that there is an inverse relationship between nitrogen content of the soil and sucrose content of the sugar beets. N_0 and N_1 were significantly higher in sucrose than N_2 . There is little tendency for nitrogen to interact significantly with other treatments.

The over-all effect of coarseness shows that the finer the pulp particles, the more sucrose is extracted. Each of the four degrees of coarseness studied gave significant differences between them.

The effect of process indicates that the processes studied are not equally efficient in the extraction of sucrose from sugar beet pulp. P_2 , P_3 and P_4 all gave highly significant increases over P_1 . P_4 is significantly higher than P_2 .

The over-all effect of time shows that greater amounts of sucrose were

extracted as the length of the digestion period was increased. Highly significant differences were obtained between all time intervals except between T_3 and T_4 , where no significant increase occurred.

Six of the 10 two-way interactions were highly significant, while one of them was significant at the .05 level.

Freezing of samples offers considerable promise in sucrose analysis not only for the purpose of storage for delayed analysis, but also as a treatment to ensure complete extraction of sugar in less time when coarse pulp is used.

Cold digestion is of no value in sucrose analysis except when carried out with completely macerated or rasped pulp. The Sachs-LeDocte aqueous digestion at 55° C. requires a fairly fine or frozen pulp or the digestion must be carried out for at least an hour to ensure equilibrium.

The Pellet method of sucrose analysis at 80° C. is superior to the 55° C. digestion when coarse unfrozen pulp is used.

The coarseness of pulp does not have much effect upon sucrose analysis when the pulp is frozen or when hot digestion is used, providing the time of digestion is adequate.

Within certain limits, the length of digestion period is not an important factor when the rasped pulp is used or when the coarser pulp is frozen, although 30 minutes should perhaps be the minimum digestion time under any conditions. If unfrozen, coarse pulp is used, the minimum digestion time should be extended to about 1 hour. Even under these conditions, the Pellet method at 80° C. is the most reliable.

The Sachs-LeDocte method should be employed where rapidity rather than highest accuracy is required. Rasped or completely macerated pulp,

analyzed by cold digestion, will be more rapid, convenient and sufficiently reliable where this method can be used.

The Pellet method should be used when emphasis is placed on accuracy and precision. The recommended 30-minute digestion at 80° C. is adequate when the beet pulp is not too coarse, but 1 hour will ensure more complete extraction with coarse, unfrozen pulp. Under some conditions the 55° C. digestion may be superior to prevent the breakdown and extraction of optically active substances and other impurities.

A further analysis of variance will be run on the data from this research in order to break down the high order interactions which will allow more specific recommendations.

A comparison study was made of saccharimetry versus copper reduction for sucrose analysis of sugar beet pulp from 50 sugar beets. The saccharimetry method gave an over-all average increase in sucrose of 0.60 per cent. The average deviations between duplicate determinations from the same beet are about equal and show good precision for both methods. The results from this study and others reported herein, indicate that while the Pellet method gives a relatively high degree of precision, its absolute accuracy in sucrose analysis may be in doubt due to the presence of other optically active constituents. Additional studies should be made to determine the cause of such consistent but variable increases, and the correlations that may exist between these increases and the relative concentrations of optically active impurities in the beets.

From the study to determine the relative size of sugar beet pulp particles which were prepared by the four methods, it has been shown that the pulp particles from the Hobart food cutter are uniform in size for either

of the chopping periods studied. It is also evident by comparing the average per cent total dry weight of total wet weight that chopping, even for 10 minutes, leaves more intact cells and causes less separation of juice and pulp than the rasp or the meat grinder.

The study of sucrose content variations due to sampling beets by quarters indicates that when an adequate number of beet quarters are bulked, processed and mixed thoroughly, a representative sample of all the beets is obtained. However, when only one beet or a small lot of beets is to be analyzed separately, sampling by quarters may not give an adequately representative sample due to natural sucrose content variations within the beets. A smaller portion of the beet, such as is removed by the rasp, would be subject to even more error and deviation from the true average composition of the beet.

This problem should be studied further to determine if there are any correlations between the position of the beet in the field in regard to sunlight, fertilization placement, irrigation furrow, etc., and variations in its sucrose content.

In addition to the above suggestions for further study in the area of sucrose analysis, the author lists the following:

1. Effects of freezing and coarseness of sample and effects of temperature and length of time of digestion on the extraction of optically active non-sugars in the beet pulp, should be determined.
2. Effects of these optically active impurities should be studied from beets grown under various environmental conditions.
3. The usefulness of freezing as a treatment to facilitate more rapid and complete sucrose extraction from non-rasped sugar beet pulp

should be investigated.

4. The Hobart food cutter as a means to obtain a more representative sample and the advantage of leaving many of the plant cells intact should be evaluated in relation to present sampling methods.

5. The problem of volume correction for marc should be evaluated objectively to attempt (or establish) an agreement as to the definition of marc and juice when lead subacetate is added.

6. The copper reduction and single saccharimetry methods of sucrose analysis should be studied further to determine the relative accuracy and precision of each.

LITERATURE CITED

- (1) Anonymous. Progress report on determination of the variation of constituents in a sufficient number of individual sugar beets for investigation of processing qualities of sugar beets. (Unpublished) Utah State Agric. College and U. S. Dept. of Agric. Dec. 31, 1955.
- (2) Anonymous. Progress report on determination of the variation of constituents in a sufficient number of individual sugar beets for investigation of processing qualities of sugar beets. (Unpublished) Utah State Agric. College and U. S. Dept. of Agric. Dec. 13, 1956.
- (3) Bachler, F. R. The Sachs-LeDocte method. Facts About Sugar 29(6): 191-194. 1934.
- (4) Bennett, J. Do beet cassettes retain sugar? Facts About Sugar 33(4): 36. 1938.
- (5) Browne, C. A. Report on sugars and sugar products. Jour. Assoc. Off. Agr. Chem. 18(2):157-162. 1935.
- (6) Browne, C. A. and Zerban, F. W. Methods of simple polarization. 3rd ed. New York: John Wiley and Sons, Inc., 1948.
- (7) Corman, C. E. Delayed sucrose analysis of pulped beet samples. Amer. Soc. Sugar Beet Tech. Proc. 541-543. 1950.
- (8) Fort, C. A. and Stout, Myron. Comparative composition of different parts of the sugar beet root. Amer. Soc. Sugar Beet Tech. Proc. 651-659. 1948.
- (9) Gaskin, J. G. N. and Hands, G. C. Precipitate error in polarizations. Sugar 49(6):60. 1954.
- (10) Goodban, A. E., Stark, J. Benjamin, and Owens, H. S. Amino acids--content of sugar beet processing juices. Agr. and Food Chem. 1(3): 261-264. 1953.
- (11) Haddock, J. L. Nitrogen requirement of sugar beets. Amer. Soc. Sugar Beet Proc. 159-164. 1952.
- (12) Honig, Pieter. Principles of sugar technology. New York: Elsevier Pub. Co., 1953.
- (13) Hungerford, E. H. and Koontz, C. R. Factors affecting the determination of sugar in beets by the Sachs-LeDocte method. Amer. Soc. Sugar Beet Tech. Proc. 303-309. 1954.

- (14) Maclay, W. D., et al. Investigations of factors influencing the Sachs-LaDocte Method. Amer. Soc. Sugar Beet Tech. Proc. 298-302. 1954.
- (15) McGinnis, R. A. ed. Beet-sugar technology. New York: Reinhold Pub. Corp., 1951.
- (16) Moore, Somers and Hallbeck, R. E. Determination of sugar in beets-- a rapid modification of the Sachs-LaDocte method. Sugar 45(10):36-39. 1950.
- (17) Nakhmanovich, J. I. and Berman, S. L. Decolorizing sugar solutions for analysis. Facts About Sugar 24(45):1142-1145. 1929.
- (18) Official Methods of Analysis of the Association of Official Agricultural Chemists. P.O. Box 540, Benjamin Franklin Station, Washington, D. C. 7th ed. 1950.
- (19) Osborn, S. J. Volume of sugar beet marc and its adsorptive effect. Ind. Eng. Chem., Anal. Ed. 6:37-40. 1934.
- (20) _____ Some details of the hot water digestion method for the determination of sugar in cassettes. Amer. Soc. Sugar Beet Tech. Proc. 548-557. 1946.
- (21) Osborn, S. J. and Brown, R. J. The marc volume of sugar beets. Facts About Sugar 27:434-435. 1932.
- (22) Silved Wedge, The. The sugar beet in the United States. United States Beet Sugar Assoc. Washington, D. C. 1936.
- (23) Smith, Paul M. Beet laboratory pulp mixer. Amer. Soc. Sugar Beet Tech. Proc. 537-538. 1946.
- (24) Thorpe's dictionary of applied chemistry. 4th ed. New York: Longmans, Green and Co., 1938.
- (25) Von Loesecke, Harry W. Outlines of food technology. New York: Reinhold Pub. Corp., 1942.
- (26) Wooley, D. G. Rood yields, sucrose, and glutamic acid content of sugar beets as influenced by soil moisture, nitrogen fertilizer, variety and harvest date. (M. S. Thesis. Dept. of Agronomy) Utah State Agricultural College, 1956.