# The Variability of Sugar Beet Constituents as Influenced by Year, Location, Variety, and Nitrogen Fertilization[1]

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## Introduction

The wide variability found in sugar beet constituents concerns farmers, processors, and researchers. An understanding of this variability and what causes it is essential to the continued profitable production of sugar beets and the extraction of the maximum amount of sugar. The reasons for this variability are only partially understood as the majority of investigators have considered single factors which can only give partial explanations. The purpose of this study was to determine the effect of soil, fertilization, climate, and variety upon the following constituents: marc, sucrose, total nitrogen, amino nitrogen, total glutamate, total anionic constituents, moisture, malic acid, oxalic acid, raffinose, and galactinol. Beet weight and beet girth measurements were also obtained and reported.

## Review of Literature

The economic value of sucrose has focused attention on the amount of sugar in beets. McGinnis (12) sampled 50 consecutively grown beets from one row and found sugar content varying from 4.4 to 16%. The sugar content of 48 samples of seven beets each varied from 12.1 to 17.3%.

Owens et al. (13) found the marc content of 15 varieties of beets grown under the same conditions varied from 3 to 6%. He found a positive correlation between marc, araban, and sucrose contents in inbred beets. Araban in the marc varied so much that it may be subject to control by plant breeding.

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Glutamic acid values usually have an inverse relation to percent sugar. Walker et al. (18) sampled beets from fields having different fertilizer levels and determined glutamic acid values ranging from 0.03 to 0.44%. Glutamic acid underwent a geometric increase with added nitrogen fertilizer. Hac et al. (9) reported the coefficient of variation for glutamic acid to be about twice as large as that for root weight and five times as large as that for sugar content.

The influence of nitrogen fertilizer upon percent sugar has been studied by many. Gardner and Robertson (6) reported a decrease in sugar percentage when nitrates were present in excess of the amount which could be used by the beets before harvest. Beet yields increased with increased nitrates but the percent sugar decreased until the maximum sugar yield was reached. Haddock (10) reported that sucrose and purity percentages were increased by heavy, frequent irrigations and a deficiency of available nitrogen. On the other hand, light irrigations and heavy nitrogen fertilization depressed sucrose and purity percentages. Haddock et al. (11) indicated that the glutamine and ammonia nitrogen components appeared to be most highly related to changes in sugar beet quality. They believed that glutamine nitrogen was of highest significance in quality variation. The concentration of nitrogen in this form was ten times that in the ammonia fraction.

Dahlberg (5) collected 24-hour composite cossette samples from ten suagr beet factories ranging in location from Kansas to Canada. Analyses of these samples gave the following results: ash content varied from 2.44 to 4.19%, nitrogen from 0.616 to 1.149%, $P_2O_5$ from 0.221 to 0.335%, and $K_2O$ from 0.86 to 1.73%. From these analyses and that of hundreds of other beets, Dahlberg concluded that ripe beets of high sugar content and purity normally have the following characteristics: low ash, low total nitrogen, low nitrate, low sodium content and a CaO plus MgO content equal to 18% or more of the soluble ash.

Rounds et al. (14) attributed variations in raffinose content to varieties rather than to the effects of nitrogen fertilization. They obtained low correlation coefficients with raffinose vs. nonsugars. McGinnis (12) described raffinose as a nearly chemically unreactive sugar which seems to pass through all the processing operations and into the molasses. It was markedly more unreactive than sucrose or other di or tri saccharides.

Swink and Finkner (17) found an inverse relation between weight of beets and galactinol content.

Total anionic constituents, malic acid, and oxalic acid are not reported in recent articles on the constituents of sugar beets; however, these constituents were determined to see if they were related to or affected the sugar content of beets.

## Materials and Methods

### *Experimental Plantings*

*North Logan, Utah — 1954:* Sugar beets were planted April 15, on the Greenville Experimental Farm, North Logan, Cache County, Utah, and harvested October 20. This was a pilot planting made to develop procedures that would be used in the main study. Segmented seed of the variety US 22 Improved was seeded at a rate of seven pounds per acre and the resulting stand was thinned to an in-row spacing of 12 inches. Seven adjacent rows 24 feet long and 22 inches apart made up each plot. The Millville silt loam soil received 100 pounds $P_2O_5$ and 80 pounds N during the year. The plot area received 26 irrigations for a total of 31.7 inches of water plus 6.09 inches of natural precipitation during the growing season.

*North Logan, Utah — 1955:* Sugar beets were planted April 30, on the Greenville Experimental Farm, and harvested October 29. Regular seed of variety *A* (SP 53104-0) was used. A Planet Jr. hand seeder was used to sow 15 pounds of seed per acre. The beets were thinned by hand at the 4-leaf stage on June 8 to an in-row spacing of 12 inches. There were four plots each consisting of seven 60 foot rows 20 inches apart.

The soil, Millville silt loam, had a pH of 7.9, organic matter content of 2.4%, total soluble salts 0.03%, $P_2O_5$ 59 pounds per acre, $K_2O$ 130 pounds per acre, lime 44 to 50%, and a mechanical analysis of 26% sand, 58% silt, 16% fine clay, and 21% clay. Texture, pH, lime content were similar to a depth of 6 to 8 feet. Organic matter drops off rapidly with depth. Previous crops on this field were: 1950 — tomatoes which received 200 pounds of treble superphosphate (46% $P_2O_5$) per acre, 1951 — grain, 1952 — alfalfa and barley, 1953 — alfalfa, and 1954 — potatoes.

All plots received 100 pounds of available $P_2O_5$ per acre, broadcast on April 20, 1955, half of the plots were side-dressed on May 2 with 80 pounds of N (ammonium sulfate) per acre and with 125 pounds of N. The latter half received an additional 125 pounds of N per acre side-dressed on July 22, 1955.

The beets were irrigated nine times receiving 16 inches of water plus 6.72 inches of natural precipitation.

*North Logan, Utah—1956:* Beets were planted May 1, on the Greenville Experimental Farm, and harvested November 2 and 3. Variety *B* was planted at a per acre rate of 15 pounds. The plots were thinned to an in-row spacing of 12 inches on June 8 and 9. There were 12 plots each 11.7 feet × 35 feet. Each plot consisted of 7 rows spaced 20 inches apart and 35 feet long.

The soil, Millville silt loam, had a pH of 8.0, total soluble salts less than 0.02%, and available $P_2O_5$ 55 pounds per acre. Lime and available $K_2O$ were not determined as previous analyses had shown these to be plentiful. Previous crops on this field were: 1950—canning peas which received 100 pounds $P_2O_5$ followed by alfalfa, 1951—alfalfa (2 crops), 1952—alfalfa (2 crops), 1953—potatoes which received 100 pounds $P_2O_5$.

All plots received 200 pounds per acre of treble superphosphate (45% $P_2O_5$) broadcast and harrowed in before planting. Four plots received no additional N, four received 80 pounds per acre of N (ammonium sulfate) May 19 side-dressed 3 inches from the beets and 3-inches deep. The remaining four plots received in like manner 125 pounds of N per acre on May 19 and an additional 125 pounds per acre of N on July 22.

Eight irrigations of from 2 to 3 inches of water each were given. Natural precipitation amounted to 4.71 inches.

*College Ward, Utah—1955:* Beets were planted May 20, on the Willy Speth farm, College Ward, Cache County, Utah, and harvested on October 15. A hand drill was used to plant 16 pounds of seed per acre of varieties *A* and *B*. Seedling emergence began May 23 and beets were hand thinned at the 4-leaf stage on June 15 to an in-row spacing of 12 inches. There were four plots each consisting of seven 60 foot rows 20 inches apart.

The soil was classified as Trenton silty clay and had a pH of 8.1, organic matter 4.2%, total soluble sales 0.09%, $P_2O_5$ 76 pounds per acre, $K_2O$ 800 pounds per acre, and a mechanical analysis of 8% sand, 43% silt, 49% fine clay, and 69% clay. Previous crops on this field were: 1951—winter wheat, 1952—winter wheat, 1953—winter wheat, 1954—corn which received 20 tons of manure per acre.

All plots received 100 pounds of available $P_2O_5$ per acre broadcast before seeding. Half of the plots received 80 pounds of N and the other half received 125 pounds per acre at seeding time and an additional 125 pounds of N per acre side-dressed on July 25.

The beets were irrigated five times receiving approximately 4 inches of water per application. Precipitation data from Utah

State University, the nearest station (5 miles away), showed that 6.46 inches of rain fell.

*Davis, California — 1955:* Beets were planted March 17, on the Campbell Tract, California Experiment Station, Davis, California, and harvested September 28. A commercial drill was used to seed variety *A* only. Due to dry soil a light irrigation was given on March 19 and excellent emergence resulted. The beets were hand thinned to an in-row spacing of 12 inches on April 28 at the 4-leaf stage. There were four plots with each plot consisting of 4 beds, 2 rows per bed, 60 feet long with a 25 × 14-inch row spacing.

The soil, Yolo loam, had a pH of 7.5, organic matter 1.3%, total soluble salts 0.04%, $P_2O_5$ 37 pounds per acre, $K_2O$ 270 pounds per acre, and a mechanical analysis of 40% sand, 44% silt, 16% fine clay, and 23% clay. Previous crops on this field were: 1951 — sugar beets, 1952 — dryland barley, 1953 — dryland barley, and 1954 — field beans.

All plots received 100 pounds of N per acre (ammonium nitrate) side-dressed 2-inches deep and 6 inches from the row on May 12 and 80 pounds of N side-dressed 2-inches deep and midway between beds on June 30.

Ten irrigations were applied with the soil wetted to a depth of 3 to 4 feet each time and at no time did the beets wilt. Natural precipitation recorded at Davis totaled 4.12 inches of which 2.16 inches fell in April.

*Saginaw, Michigan — 1955:* Beets (Variety A) were planted April 16, two miles west of Saginaw, Michigan, and harvested October 24. They were blocked and thinned on May 27 with a 90% stand reported on June 6. The plot consisted of seven rows, spaced 28 inches apart and 60 feet long.

The soil, Kawkawlin loam, had a pH of 7.2, organic matter 4.6%, total soluble salts 0.04%, $CO_2$ soluble $PO_4$ 1 ppm, and $CO_2$ soluble $NO_3$ 3 ppm. Previous crops on this field were: 1952 — white beans, 1953 — wheat, and 1954 — white beans. One-hundred fifty pounds of 3-12-12 fertilizer per acre had been applied each of these three years.

The plot received 500 pounds of 6-24-12 fertilizer per acre applied 2 inches below the seed at planting time. On June 4 the beets were side-dressed with 40 pounds of liquid nitrogen per acre.

These beets were grown without irrigation but 20.56 inches of rain fell during the season.

### *Harvesting and Processing Procedures*

*1954:* Fifty beets were sampled at random from the center three rows of the seven-row plot and refrigerated at 33° F. Three days later the refrigerated beets were washed with cold water, numbered, weighed, and refrigerated at 33° F. During November the beets were rasped and aliquots frozen for chemical analyses.

*1955:* Fifty beets were sampled from the center three rows (four rows in California) of each plot as follows: a 5-foot length was discarded from each end of each plot; the remainder of the plot was divided into five equal sections; and a 10 beet sample, made up of five random pairs of beets, was taken from each section. In order for a beet to have been included in the sample, it must have had an adjacent in-the-row mate and both must have been free from adjacent skips and/or multiple beets. The beets were lifted with a garden fork, labeled, topped, and placed in an ice water bath.

All beets were topped by removing the petioles by hand and rubbing the crowns with an ordinary plastic household scouring pad. This left the crown on each beet but removed most of the green material as well as the scar tissue. As soon as the beets were topped they were placed in ice water (in 55 gallon drums) and held at 0° C while they were transported to the laboratory.

The beets were cleansed with cold water, weighed to the nearest 1/10 pound, and classified into one of four shape categories. The maximum girth of each beet was determined. The whole beets were individually chopped in a Hobart Food cutter (Model T 215) as the bowl rotated for approximately 2 minutes. Twelve 10-gram samples of the macerated material were weighed out from each beet, wrapped in aluminum foil, and frozen immediately. The samples were held at −5° F to await chemical analysis. The interval between digging and completion of processing was approximately 3 hours.

*1956:* Instead of selecting five random pairs of beets from each section as in 1955, 10 beets were selected by complete randomization within each section. Fifty beets were thus harvested from each plot.

### *Chemical Methods and Procedures*

*Sample Preparation:* The frozen, aluminum-foil wrapped samples of chopped beets were weighed on an analytical balance, the frozen sample removed to a 250 ml beaker and the foil weighed to get the net weight of the sample. As many individual determinations as possible were made on each individual sample.

Duplicate determinations were made on two or more separate samples of the same beet.

The extraction procedure was a modification of that used by Goodban, Stark, and Owens (7). Fifty ml of boiling distilled water were added to the beaker which was then placed on a variable temperature hot plate and maintained between 78° and 82° C for 30 minutes. Each beaker contained a thermometer and was covered with a watch glass. At the end of the 30 minute period the water was carefully decanted into a 100 ml volumetric flash, another 50 ml portion of hot distilled water was added to the pulp, washing down any material adhering to the side of the beaker, and heating was repeated for another 30 minutes. This portion was decanted into the volumetric flask at the end of the time interval. Small portions of hot water were added to the pulp and decanted into the volumetric flask until the liquid reached the mark. Usually the evaporation loss amounted to 10 to 20 ml. On cooling to room temperature the volume was finally adjusted to 100 ml using distilled water.

*Chemical Procedures:* The salts of oxalic and malic acid in the sugar beet extract were converted to acids by passing some of the extract over a Dowex 50 hydrogen cation exchange resin and the acids were then absorbed on a Dowex 1 anion exchange resin. The acids were then fractionally eluted with 30 ml of 0.35 N ammonium carbonate. Lactic and glycolic acids were eluted first and discarded. Malic acid was determined by a slight modification of the method of Goodban and Stark[3] as outlined by Stark, Goodban and Owens (16) page 564.

Oxalic acid was then eluted from the resin with 50 ml of 0.55 N ammonium carbonate. The eluate was made acid with 2.5 ml of concentrated sulfuric acid, 1 ml of 1% manganese sulfate was added, and the solution titrated with 0.005-0.01 N potassium permanganate. The end point was reached when 0.15 ml of the potassium permanganate produced a color which persisted for at least 10 seconds. Citric acid, which was present, was oxidized very slowly by the permanganate at the temperature of the titration.

Sucrose was determined by the method found in the Association of Official Agricultural Chemists (2) pages 347, 348 and 507.

Total nitrogen was determined by the procedure found in the Association of Official Agricultural Chemists (1) page 13.

[3] Private communication from Dr. H. S. Owens, deceased, formerly supervisor, Sugar Beet Unit, Field Crop Utilization Section, Western Utilization Research Branch, U. S. Department of Agriculture.

Amino nitrogen was isolated on Dowex 50 Cation exchange resin and estimated by the ninhydrin method as modified by Cocking and Yemm (4).

Total glutamate was determined by the microbiological method of Hac, Walker, and Dowling (9) and Hac, Long and Blish (8).

Total anionic constituents were determined by the conversion of salts to acid form on Dowex 50 cation exchange resin followed by titration with sodium hydroxide.

In determining marc the ground samples were extracted with 80% alcohol, dried, and weighed.

In determining raffinose and galactinol, the frozen samples were partially thawed, then pressed at 9,000 pounds per square inch in a Carver laboratory press (Serro and Brown, 15). The expressed juice was used directly or diluted with water to bring the sample within range of the standards.

A special drying-developing oven was constructed essentially according to plans supplied by Robert J. Brown of the Great Western Sugar Company, Denver, Colorado, (Drawing No. 9419).

The minor modifications of the basic methods were these: raffinose: (a) Standards were made up to give 4, 8, 12, and 16 micrograms in a 10 microliter spot. It was necessary to use a sucrose base for these standard solutions. (b) An n-propyl alcohol-ethyl acetate-water was used as a solvent mixture for about 22 hours. (c) the $\alpha$ napthol solution was applied to the paper by drawing the paper quickly through the solution in a tray and immediately hanging it up to dry. This gave very even distribution without streaking the raffinose spots. Galactinol: (a) Standard spots contained 2, 4, 8, and 12 micrograms. The galactinol used as standard was furnished by Mr. Robert J. Brown of the Great Western Sugar Company.

## Results and Discussion

Chemical analyses of the beets produced in 1954 are summarized in Table 1. The high variability due to determinations was believed to be due to inherent limitatons of the analytical methods, limited experience of the analvsts, long sample storage period during which temperature controls were not adequate, inherent variability of the sugar beets, and the low concentration of some of the constituants in the beets. Correlation coefficients between sucrose and total nitrogen and between sucrose and total anionic constituents were nonsignificant.

It should be remembered that this was a preliminary trial used to develop procedures to be used in the major trials of 1955 and 1956.

Table 1.—The mean, and coefficient of variation values for the constituents of the 50 sugar beets sampled in Utah in 1954.

| Constituent | | Mean | Coefficient of variation | |
|---|---|---|---|---|
| | | | Single beet[1] | Single determinations[2] |
| Glutamate | % fresh weight | 0.1240 | 75.4 | 23.8 |
| Reducing sugar | % fresh weight | 0.17 | 48.2 | 15.2 |
| Amino nitrogen | % fresh weight | 0.0184 | 44.6 | 14.1 |
| Anionic constituents | meq/100g of sucrose | 29.16 | 22.2 | 7.0 |
| Total nitrogen | % fresh weight | 0.1860 | 18.3 | 5.8 |
| Total marc | % fresh weight | 5.62 | 13.3 | 4.2 |
| Dry matter | % fresh weight | 25.44 | 7.4 | |
| Sucrose | % fresh weight | 17.92 | 6.6 | 2.1 |

[1] The standard error of a single beet, eliminating determination error and assuming randomized sampling, as a percent of the mean. Coefficient of variation of single beets.

[2] The standard error of a single determination expressed as percent of the mean. Coefficient of variation of single determinations.

The results of the analyses of the beets produced in 1955 and 1956 are summarized in Table 2. Mean comparisons can be made with the 1954 data but it is difficult to draw conclusions because the constituents reported and the units of measurement differed. Direct comparisons may be made between coefficients of variation. Glutamate was the most variable and sucrose the least variable constituent in both years.

Other constituents differ in rank but were essentially in the same order. The 1956 results represent 50 beets of one variety sampled at one location (Utah) and grown under one nitrogen level. The results for 1955 (Table 2) repreesnt 11 lots of 50 beets each which include 2 varieties, 3 locations (Utah, California and Michigan) and 4 nitrogen levels.

*Correlations:* Simple correlation coefficients involving all possible combinations (66 in all) were calculated for each nitrogen level and location (11 in all) in 1955 and 1956. The results are summarized and presented in Table 3. Sucrose was negatively correlated with all chemical constituents except galactinol and was positively correlated with marc. No correlation was found between sucrose $\times$ beet weight, sucrose $\times$ beet girth, or sucrose $\times$ galactinol. Other significant correlations may be seen in Table 3.

The negative correlation between sucrose $\times$ glutamate is in agreement with Walker et al. (18). The negative correlations between sucrose $\times$ total nitrogen and sucrose $\times$ amino nitrogen agrees with Haddock (10).

Table 2.—Mean and coeffcients of variation for sugar beets sampled in Utah, California, and Michigan in 1955 and in Utah in 1956.

| Constituent | | Mean 1955 | Mean 1956 | Coefficients of Variation 1955 Single beet[1] | 1955 Single determinations[2] | 1956 Single beet[1] | 1956 Single determinations[2] |
|---|---|---|---|---|---|---|---|
| Glutamate | mg/100g beet | 58.7 | 59.5 | 64.1 | 20.2 | 48.2 | 15.2 |
| Galactinol | % fresh weight | .057 | .025 | 51.0 | 16.1 | 36.4 | 11.5 |
| Amino nitrogen | mg/100g beet | 37.9 | 40.0 | 41.9 | 13.2 | 43.2 | 13.7 |
| Beet weight | pounds per beet | 2.22 | 2.19 | 41.8 | | 33.0 | |
| Raffinose | % fresh weight | .112 | .172 | 29.2 | 9.2 | 30.4 | 9.6 |
| Malic acid | mg/100g beet | 41.2 | 29.7 | 27.7 | 8.8 | 29.3 | 9.3 |
| Beet girth | inches per beet | 12.6 | 11.6 | 16.0 | | 12.8 | |
| Total nitrogen | mg/100g beet | 227.5 | 216.2 | 15.9 | 5.0 | 19.9 | 6.3 |
| Oxalic acid | mg/100g beet | 109.3 | 96.6 | 15.5 | 4.9 | 16.0 | 5.1 |
| Anionic constitutents | meq/100g beet | 8.87 | 7.90 | 15.3 | 4.8 | 15.1 | 4.8 |
| Marc | % fresh weight | 4.93 | 4.90 | 9.5 | 3.0 | 10.9 | 3.4 |
| Sucrose | % fresh weight | 14.9 | 15.0 | 7.1 | 2.2 | 8.3 | 2.6 |

[1] The standard error of a single beet, eliminating determination error and assuming randomized sampling, as a percent of the mean. Coefficient of variation of single beets.

[2] The standard error of a scingl determination expressed as percent of the mean. Coefficient of variation of single determinations.

Table 3.—Tabulation of the significance of the respective correlation coefficients for the 11 different lots of beets.

| | | Number of times (out of 11) correlation coefficients were significant | | | |
|---|---|---|---|---|---|
| | | .05 level | | .01 level | |
| | Comparison | Positive | Negative | Positive | Negative |
| 1 | weight × girth | 11 | | 11 | |
| 2 | sucrose | | | | |
| 3 | marc | | | | |
| 4 | malic acid | 7 | | 3 | |
| 5 | oxalic acid | 1 | | | |
| 6 | anionic constituents | 2 | | | |
| 7 | total nitrogen | | | | |
| 8 | amino nitrogen | | | | |
| 9 | glutamate | 1 | | | |
| 10 | galactinol | | | | |
| 11 | raffinose | 1 | | | |
| 12 | girth × sucrose | | | | |
| 13 | marc | | 1 | | |
| 14 | malic acid | 7 | | 3 | |
| 15 | oxalic acid | | | | |
| 16 | anionic constituents | 6 | | | |
| 17 | total nitrogen | | | | |
| 18 | amino nitrogen | | | | |
| 19 | glutamate | 1 | | | |
| 20 | galactinol | | 1 | | |
| 21 | raffinose | | | | |
| 22 | sucrose × marc | 10 | | 5 | |
| 23 | malic acid | | 8 | | 8 |
| 24 | oxalic acid | | 9 | | 7 |
| 25 | anionic constituents | | 10 | | 8 |
| 26 | total nitrogen | | 3 | | 1 |
| 27 | amino nitrogen | | 6 | | 4 |
| 28 | glutamate | | 5 | | 4 |
| 29 | galactinol | | | | |
| 30 | raffinose | | 3 | | 1 |
| 31 | marc × malic acid | | 8 | | 5 |
| 32 | oxalic acid | 1 | 1 | | |
| 33 | anionic constituents | | 2 | | |
| 34 | total nitrogen | 3 | | | |
| 35 | amino nitrogen | | 2 | | |
| 36 | glutamate | | 3 | | 1 |
| 37 | galactinol | 1 | | | |
| 38 | raffinose | | 1 | | |
| 39 | malic acid × oxalic acid | 6 | | 2 | |
| 40 | anionic constituents | 9 | | 8 | |
| 41 | total nitrogen | | | | |
| 42 | amino nitrogen | 4 | | | |
| 43 | glutamate | 3 | | | |
| 44 | galactinol | 1 | | 1 | |
| 45 | raffinose | | | | |

TABLE 3.—Continued.

| Comparison | Number of times (out of 11) correlation coefficients were significaant .05 level Positive | .05 level Negative | .01 level Positive | .01 level Negative |
|---|---|---|---|---|
| 46 oxalic acid × anionic constituents | 10 | | 8 | |
| 47 total nitrogen | 1 | | 1 | |
| 48 amino nitrogen | 3 | | 1 | |
| 49 glutamate | 4 | | 1 | |
| 50 galactinol | 1 | | 1 | |
| 51 raffinose | 1 | | 1 | |
| 52 anionic const. × total nitrogen | 7 | | 5 | |
| 53 amino nitrogen | 6 | | 4 | |
| 54 glutamate | 5 | | 3 | |
| 55 galactinol | 1 | | | |
| 56 raffinose | 1 | | | |
| 57 total nitrogen × amino nitrogen | 10 | | 10 | |
| 58 glutamate | 10 | | 8 | |
| 59 galactinol | | 2 | | |
| 60 raffinose | | | | |
| 61 Amino nitrogen × glutamate | 10 | | 9 | |
| 62 galactinol | | 3 | | |
| 63 raffinose | | | | |
| 64 glutamate × galactinol | 2 | 3 | | 2 |
| 65 raffinose | 1 | | 1 | |
| 66 galactinol × raffinose | | 1 | | |

*Constituents and Coefficients of variation:* Table 4 gives the mean constituent value for each variety, year, location, and rate of nitrogen fertilization. The corresponding coefficient of variation for a single determination is included. Each figure is based on a 50 beet sample and duplicate determinations, except for beet weight and girth on which only single measurements were made.

The coefficient of variation for glutamate is about 1.5 times that for beet weight and 8.8 times that for sucrose. These agree essentially with Hac et al. (9) who reported multiples of 2 and 5, respectively.

Equating the coefficient of variation for sucrose equal to one and relating the other coefficients to it gives the following: (1955)

| | | | |
|---|---|---|---|
| Sucrose | 1 | malic acid | 3.8 |
| marc | 1.3 | raffinose | 4.0 |
| anionic constituents | 2.1 | beet weight | 5.7 |
| oxalic acid | 2.1 | amino nitrogen | 5.7 |
| total nitrogen | 2.2 | galactinol | 7.0 |
| beet girth | 2.2 | glutamate | 8.8 |

Table 4.—Constituents of sugar beets and coefficients of variation in relation to year, location, variety, and rate of nitrogen fertilization.

| Year | Location | Variety | Applied N per acre | Glutamate Mean mg/100g beet | Glutamate C.V.[1] | Galactinol Mean % fresh weight | Galactinol C.V.[1] | Amino nitrogen Mean mg/100g beet | Amino nitrogen C.V.[1] | Beet weight Mean pounds per beet | Beet weight C.V.[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | lb | | | | | | | | |
| 1955 | Davis, California | A | 180 | 165.6 | 18.5 | .036 | 13.1 | 46.2 | 11.1 | 3.02 | 12.5 |
| | Saginaw, Michigan | A | 70 | 85.8 | 10.9 | .040 | 10.6 | 59.0 | 9.9 | 2.51 | 11.7 |
| | College Ward, Utah | A | 80 | 24.5 | 13.4 | .082 | 18.3 | 25.9 | 15.1 | 2.20 | 11.6 |
| | | A | 250 | 24.4 | 14.4 | .057 | 9.6 | 27.7 | 14.8 | 2.25 | 13.1 |
| | | B | 80 | 32.2 | 13.0 | .056 | 18.1 | 31.1 | 12.7 | 2.53 | 13.0 |
| | | B | 250 | 35.8 | 13.2 | .056 | 11.4 | 25.5 | 16.4 | 2.13 | 15.4 |
| | North Logan, Utah | A | 80 | 49.9 | 13.6 | .059 | 18.8 | 34.5 | 13.2 | 2.01 | 11.9 |
| | | A | 250 | 84.1 | 17.6 | .076 | 11.7 | 48.9 | 10.5 | 2.05 | 13.2 |
| | | B | 80 | 29.0 | 20.3 | .057 | 21.7 | 25.0 | 19.3 | 1.75 | 13.2 |
| | | B | 250 | 64.3 | 9.4 | .051 | 11.2 | 55.7 | 13.5 | 1.79 | 16.1 |
| 1956 | North Logan, Utah | B | 80 | 59.5 | 15.2 | .025 | 11.5 | 40.0 | 13.7 | 2.19 | 10.3 |

| Year | Location | Variety | Applied N per acre | Raffinose Mean % fresh weight | Raffinose C.V.[1] | Malic acid Mean mg/100g beet | Malic acid C.V.[1] | Beet girth Mean inches per beet | Beet girth C.V.[1] | Total nitrogen Mean mg/100g beet | Total nitrogen C.V.[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | lb | | | | | | | | |
| 1955 | Davis, California | A | 180 | .044 | 7.0 | 39.1 | 10.1 | 13.5 | 5.0 | 184.1 | 6.2 |
| | Saginaw, Michigan | A | 70 | .044 | 9.3 | 30.5 | 9.6 | 13.6 | 4.9 | 289.0 | 4.0 |
| | College Ward, Utah | A | 80 | .116 | 9.6 | 49.8 | 8.2 | 12.6 | 4.7 | 185.9 | 3.8 |
| | | A | 250 | .129 | 12.1 | 48.4 | 10.8 | 12.6 | 5.4 | 205.7 | 4.1 |
| | | B | 80 | .126 | 8.8 | 48.3 | 9.0 | 13.4 | 4.7 | 208.1 | 4.5 |
| | | B | 250 | .109 | 6.4 | 46.4 | 7.9 | 12.6 | 5.1 | 204.8 | 5.0 |
| | North Logan, Utah | A | 80 | .136 | 8.0 | 32.3 | 6.0 | 12.1 | 5.2 | 234.3 | 5.4 |
| | | A | 250 | .131 | 9.1 | 48.7 | 7.4 | 12.7 | 5.1 | 280.5 | 3.1 |
| | | B | 80 | .143 | 6.8 | 29.9 | 8.5 | 11.3 | 4.9 | 188.3 | 9.4 |
| | | B | 250 | .146 | 8.6 | 39.0 | 7.4 | 11.5 | 5.4 | 294.7 | 4.7 |
| 1956 | North Logan, Utah | B | 80 | .172 | 9.6 | 29.7 | 9.3 | 11.6 | 4.1 | 216.2 | 6.3 |

[1] Coefficient of variation of a single beet assuming no determination error and completly randomized sampling.

Table 4.—Constituents of sugar beets and coefficients of variation in relation to year, location, variety, and rate of nitrogen fertilization.—Cont.

| Year | Location | Variety | Applied N per acre | Oxalic acid: Mean mg/100g beet | Oxalic acid: C.V.[1] | Anionic constituents: Mean meq/100g. beet | Anionic constituents: C.V.[1] | Marc: Mean % fresh weight | Marc: C.V.[1] | Sucrose: Mean % fresh weight | Sucrose: C.V. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | lb | | | | | | | | |
| 1955 | Davis, California | A | 180 | 121.5 | 5.3 | 8.6 | 5.1 | 4.2 | 3.1 | 13.5 | 2.7 |
| | Saginaw, Michigan | A | 70 | 101.2 | 4.8 | 8.9 | 4.2 | 4.6 | 3.2 | 14.5 | 2.8 |
| | College Ward, Utah | A | 80 | 121.0 | 4.8 | 9.8 | 4.5 | 4.8 | 3.1 | 14.6 | 2.1 |
| | | A | 250 | 122.3 | 5.0 | 10.4 | 5.2 | 4.9 | 2.8 | 14.1 | 2.9 |
| | | B | 80 | 120.6 | 4.4 | 9.8 | 4.3 | 4.9 | 2.6 | 15.0 | 1.6 |
| | | B | 250 | 120.8 | 3.9 | 9.5 | 4.0 | 5.0 | 2.8 | 14.7 | 1.5 |
| | North Logan, Utah | A | 80 | 92.3 | 5.4 | 7.7 | 5.1 | 5.3 | 3.4 | 15.9 | 1.8 |
| | | A | 250 | 118.0 | 3.8 | 10.0 | 4.3 | 5.2 | 2.7 | 15.0 | 2.0 |
| | | B | 80 | 88.9 | 6.2 | 6.3 | 5.5 | 5.1 | 3.0 | 16.0 | 2.2 |
| | | B | 250 | 98.7 | 5.1 | 7.9 | 5.6 | 5.3 | 3.1 | 15.9 | 2.4 |
| 1956 | North Logan, Utah | B | 80 | 96.6 | 5.1 | 7.9 | 4.8 | 4.9 | 3.4 | 15.0 | 2.6 |

[1] Coefficient of variation of a single beet assuming no determination error and completely randomized sampling.

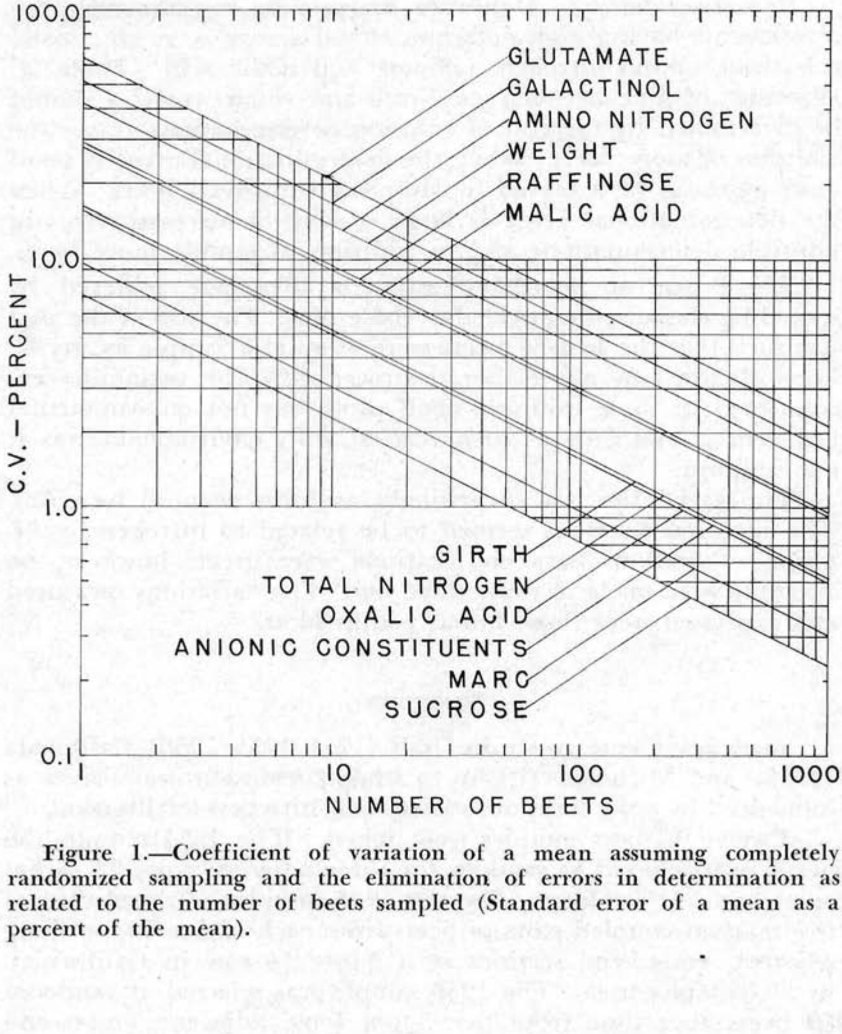


**Figure 1.—Coefficient of variation of a mean assuming completely randomized sampling and the elimination of errors in determination as related to the number of beets sampled (Standard error of a mean as a percent of the mean).**

Figure 1 graphically portrays coefficients of variation of the means assuming completely randomized sampling and the elimination of errors in determination as related to the number of beets in a composited sample. This figure may be used to predict the number of beets which must be sampled and composited before analysis if a particular coefficient is desired. For example to determine glutamate with a 10% coefficient of variation requires a composited 45 beet sample. On the other hand this sample, analysed for sucrose, would have a 1.2% coefficient of variation. Glutamate was about eight times as variable as sucrose. Other comparisons may be made.

*Recommendations:* Duplicate analyses are recommended for constituents having high determinational errors, e. g. glutamate, galactinol, amino nitrogen, raffinose and malic acid (Table 2). Drawing the line between duplicate and single analyses should be determined by the cost of running determinations versus the addition of more beets. When the determinational error is small more gain can be achieved by sampling additional beets. When the determinational error is large it may be necessary to run multiple determinations and in addition to sample more beets.

There was no significant gain or advantage achieved by spreading the sampling over the whole plot. The size of the plot was such that the first 50 beets were as good a sample as any 50 beets obtained by any elaborate procedure. The variability encountered in these uniform small plots was not environmental but genetic and only as such reacted with environment was it not uniform.

The variability was surprisingly uniform over all locations. The nitrogen fractions seemed to be related to nitrogen fertilization. Variations between locations were great; however, no attempts were made to draw these out. The variations measured and expressed were those found within plots.

## Summary

Sugar beets were grown in Utah (1954, 1955, 1956), California (1955), and Michigan (1955) to study constituent variability as influenced by year, location, variety and nitrogen fertilization.

Twelve 50 beet samples were taken. The 1954 sample (50 beets) was selected at random for three adjacent rows 22 inches apart and 24 feet long. The ten 1955 samples were selected as five random sampled pairs of beets from each of five 10-foot long adjacent, end-to-end sections of a 3-row (4 row in California) by 50-foot plot area. The 1956 sample was selected at randoom 10 beets at a time from five 5-foot long, adjacent, end-to-end sections of a 3-row by 25-foot plot area.

Each beet was lifted, topped, labeled, and placed in ice water while transported to the laboratory. At the laboratory each beet was cleaned, weighed, measured, chopped, sampled, and frozen independently. About 3 hours clapsed between digging and sample freezing.

Laboratory analyses made on the basis of fresh weight included: mg/100g for glutamate, amino nitrogen, total nitrogen, malic acid and oxalic acid: meq/100 g for anionic constituents; and percent for galactinol, raffinose, marc, and sucrose. Duplicate determinations were made on each test for each constituent.

Statistical analyses of the data gave negative correlations between sucrose and the other chemical constituents except galactinol. Sucrose and marc were positively correlated but no relationship was evident between sucrose × beet weight and sucrose × beet girth. Sucrose was the only constituent which was consistently changed in the same direction (in this case downward) by an increase in nitrogen fertilization. The nitrogen constituents (glutamate, amino nitrogen and total nitrogen) were inconsistent in this respect.

Changes in coefficients of variation were graphically portrayed in relation to the number of beets in a composited sample. Using this graph one may predict the number of beets which must be sampled and composited before analysis if a particular coefficient of variation for any constituent is desired. Glutamate was the most variable and sucrose the least variable constituent measured. Varietal differences were not consistent and were usually not great.

Large determinational errors suggest a need for duplicate analyses and the addition of more beets whereas only single analysis may be needed where small determinational errors are encountered.

## Literature Cited


(1) ADAC Methods of Analysis. 1950. 7 Ed. 2.25, p. 13.

(2) ADAC Methods of Analysis. 1950. 7 Ed. 22.32, 22-33, 29.36, 29.39, and 29.38. pp. 347, 348, and 507.

(3) ADAC Methods of Analysis. 1950. 7 Ed. 29.2, p. 493.

(4) Cocking, E. C., and Yemm, E. W. 1954. Estimation of amino acid by ninhydrin. Biochem. J. 58: xii.

(5) Dahlberg, H. W. 1950. Composition of beets grown in widely separated areas Proc. Am. Soc. Sugar Beet Technol. VI: 565-569.

(6) Gardner, R. and Robertson, D. W. 1942. Nitrogen requirements of sugar beets. Colorado Exp. Sta. Tech. Bull. 28.

(7) Goodban, A. E., Stark, J. B., and Owens, H. S. 1953. Amino acids—content of sugar beet processing juices. Agr. and Food Chem. 1: 261-264.

(8) Hac, L. R., Long, M. L., and Blish, M. J. 1949. The occurrence of free L-glutamic acid in various foods. Food Technol. 3: 35.

(9) Hac, L. R., Walker, A. C., and Dowling, B. B. 1950. The effect of fertilization on the glutamic acid content of sugar beets in relation to sugar production. Proc. Am. Soc. Sugar Beet Technol. VI: 401-411.

(10) Haddock, J. L. 1953. Sugar beet yield and quality. Utah Agr. Exp. Sta. Bull. 362.

(11) Haddock, J. L., Linton, D. C. and Hurst, R. L. 1956. Nitrogen constituents associated with reduction of sucrose percentage and purity of sugar beets. J. Am. Soc. Sugar Beet Technol. IX (2): 110-117.

(12) McGINNIS, R. A. 1951. Beet-sugar Technology. Reinhold Publishing Corporation, New York.

(13) OWENS, H. S., McCOMB, E. A., and DEMING, G. W. 1954. Composition and percentage of marc in some varieties of inbred sugar beets. Proc. Am. Soc. Sugar Beet Technol. VIII: 257-271.

(14) ROUNDS, H. G., RUSH, G. E., OLDEMEYER, D. L., PARRISH, C. P. and RAWLINGS, F. N. 1958. A study and economic appraisal of the effect of nitrogen fertilization and selected varieties on the production and processing of sugar beets. J. Am. Soc. Sugar Beet Technol. X: 97-116.

(15) SERRO, R. F., and BROWN, R. J. 1954. Improved chromatographic method for analysis of sugar beet products. Anal. Chem. 25: 890.

(16) STARK, J. B., GOODBAN, A. E., and OWENS, H. S. 1953. Beet sugar liquors—determination and concentration of lactic acid in processing liquors. Agr. and Food Chem. 1: 564-565.

(17) SWINK, J. F., and FINKNER, R. E. 1956. Galactinol-weight relationship in breeding for resistance to the sugar beet nematodes. J. Am. Soc. Sugar Beet Technol. IX: 70-73.

(18) WALKER, A. C., HAC, L. R., ULRICH, A. and HILLS, F. J. 1950. Nitrogen fertilization of sugar beets in the Woodland area of California. I. Effects upon glutamic acid content, sucrose concentration, and yield. Proc. Am. Soc. Sugar Beet Technol. VI: 362-371.