Composition and Percentage of Marc in Some Varieties of Inbred Sugar Beets

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Recently, development of new varieties of sugar beets with improved processing characteristics has been emphasized to a greater extent than previously when increased disease resistance was the important factor. Marc content, however, has received little attention. To add to the knowledge of that part of the beet and to learn something about its genetic character, this laboratory and the Sugar Plant Investigations Division initiated preliminary work with 15 varieties of inbred beets. These were analyzed for sucrose, marc, pectin, araban, and galactan.

The importance of these components of the beet are summarized as follows. The marc content of sugar beets partly controls the volume of solution of basic lead acetate used in the Sachs-Le Docte method of sucrose analysis. Thus it plays a significant role in determining materials balance *in* the beet factory and in determining payments to the beet grower. The constituents of marc are important in controlling the maximum temperature at which the diffusers can operate. In factories which have pulp presses, the pulp is maintained in a pressable condition and the extraction of excessive amounts of materials, which may have deleterious effects on later operations, is prevented by keeping the temperatures low in the diffusers. Unfortunately, this may lead to fermentation and all its problems related to loss of sucrose and production of lactic acid, dextran and other harmful fermentation products.

The literature is replete with measurements of marc volume (l)[3], but the values range from 0.6 to 2.4 ml. per normal weight (26 grams) of beets. Insufficient study has been made of composition of marc (2). Because of the importance of marc content and its composition the investigation of these factors was undertaken.

Preparation and Analysis of Samples

Inbred beets were grown in plots at Fort Collins, Colorado, during the 1952 season. One sample of beet cossettes was collected from a factory in California, in order to have marc of a typical commercial variety for comparative purposes. The inbreds were rasped and composites of the pulp from nine or ten roots were made. Aliquots of the composites were used for sucrose analysis. Other aliquots were immediately frozen in pliofilm bags and air expressed to the Western Regional Research Laboratory. The frozen samples were kept at —20° F. until analyzed. The varieties are described in Table I.

Enzymes in the marc were denatured by adding about 100 g. of weighed, frozen marc directly to 2.5 volumes of boiling 95 percent alcohol. The

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[3] Numbers in parentheses refer to literature cited.

Table 1.—Description of the Beet Varieties Used in this Study.

1952 No.	Origin	Generations
77	Cross; sugar and globe garden, short white beet, slightly pink skin.	G5 (sssg)[1]
81	Same as No. 77. Near sugar beet shape	G5 (ssgg)
89	Commercial	G8 (sggssssg)
99	"Pioneer" commercial sugar beet	G10 (ssgssgsssg)
115	Probably "Fredricksen" sugar beet	G8 (ssgsssgg)
119	"Eagle Hill" (Danish) sugar beet	G5 (ssssg)
123	"Cesena" (Italian) sugar beet	G5 (ssssg)
124	"Hilleshog" (Swedish) sugar beet	G5 (ssssg)
144	Commercial sugar beet	G9 (o.p. sgsgsssg)
159	Commercial sugar beet	G7 (ssgsssg)
170	"Pioneer" sugar beet	C7 (ssgsssgg)
188	"Pioneer" sugar beet	G9 (ssgsgsgsg)
201	"Flat Foliage" sugar beet	G9 (sgsgsgsgsg)
238	Commercial sugar beet	G8 ----------
246	"Flat Foliage" sugar beet	G10 ----------

[1] Capital G and number indicate number of generations of known genetic history, "s" indicates space isolated or bagged plant; "g" indicates sib pollination.

marc was kept in boiling alcohol 10 minutes after thawing and then was extracted in a Soxhlet extractor. Five hundred ml. of 70 percent ethanol was used in the pot. The extractor held 330 ml; therefore, the concentration of the alcohol in the latter was never less than 80 percent in order to decrease dissolution of the araban. Not less than 15 extractions were performed but this number resulted from the convenience of overnight extraction and no significance should be attached to it. No arabinose has been found in the acid-treated extraction liquors, indicating that only a small amount of araban, if any, was lost during the extraction. No sucrose detectable by oc -naphtol remained in the pulp. The extracted pulp was dried *in vacuo* at 60° C. overnight. Moisture determinations were run by another overnight drying *in vacuo* at 60° C. All results are calculated to moisture-free basis.

Pectin was determined by the McCready-McComb procedure (3). To obtain maximum extraction of the uronic acid-containing material, it was necessary to add 0.05 N alkali with 0.5 percent ethylene diamine tetracetate and extract for one hour before neutralization and addition of the pectinase.

Araban and galactan were run chromatographically after hydrolysis with acid for four hours. The procedure was to add 0.25 g. of pulp to 5 ml. of 1 N HC1 in a centrifuge tube. Duplicates of each sample were run. The tubes were placed in a boiling water bath and tightly stoppered after reaching temperature. After a 4-hour period they were made to the original volume with water, if necessary, and centrifuged. The clear liquid was decanted into vials. The vials were stoppered and stored in the refrigerator.

Three 1-ul spots of the acid-hydrotyzed solution were spaced 1.5 cm. apart on S 8c S 507 paper. Standards containing arabinose and galactose at 0.5, 1.0, and 2.0 percent concentrations were applied on the same paper. The carbohydrates were chromatographed with Brown's raffinose solvent (4). After an overnight development, the papers were dried at 45° C. for 40 or more minutes, dipped into 3 percent aniline in ether, then into 3

percent trichloracetic acid in ether and dried at 80° C. for 15 minutes. In the earlier experiments, the reflection density of the spots was measured, using a 515 mu filter as suggested by McCready and McComb (5) of this laboratory. Measurements of galactose spots were more sensitive at 365 mu, however, and this filter was used for later experiments. Two-way chromatograms using t-butyl alcohol, formic acid, and water (6) and phenol-water in the second direction showed insufficient glucose to interfere with the galactose measurements. The standard deviation of 13 determinations of arabinose was 1.2 percent.

Time of hydrolysis with 1 N hydrochloric acid to obtain reproducible results was not critical for araban. The same results were obtained in 4 and 6 hour treatment. The galactan appears to be incompletely hydrolyzed at the end of 4 hours, inasmuch as higher values for galactan were obtained after 24 hours' hydrolysis. A compromise was necessary, because the yield of arabinose is decreased when a 24-hour period of hydrolysis is used. A 4-hour period of hydrolysis was chosen for this work because it gave the maximum yield of arabinose and was only 10 percent low with galactose. Because of decomposition of the free sugars during hydrolysis, the results for araban and galactan will be low. The actual loss, which appears to be less than 5 percent will be determined when sufficient quantities of these materials are available for test.

Results

Table 2 summarizes the data on marc content and the percentage of araban, galactan, and anhydrouronic acid in the examined varieties of beets. There are several items of interest. The correlation between marc content and sucrose content of the different varieties is remarkably good (r == 0.69) The explanation for the correlation is not clear. Although the marc con

Table 2.—Analysis of Beets: Sucrose, Marc, and Water-Soluble Polysaccharide Contents.

1952 No.	Sucrose % on beet	Marc % on beet	A.U.A.[1] % on marc	Arabinose % on marc	Galactose % on marc
77	10.0	3.1	26	11	8
81	12.6	3.8	25	12	6
89	14.9	3.8	26	18	8
90	15.0	3.8	26	11	6
115	9.0	2.7	26	12	9
119	11.5	4.0	23	11	6
123	12.5	3.9	28	14	6
124	14.4	4.5	27	13	4
144	12.5	4.1	26	15	4
159	9.9	3.6	24	14	4
170	14.3	3.7	25	17	5
188	14.6	4.1	22	15	5
201	13.2	4.4	25	15	5
238	14.2	4.5	24	16	4
246	15.3	6.1	24	18	5
Calif. Commercial	...	5	21	22	8

[1] Anhydrouronic acid (a measure of pectin).

tent may vary by twofold, the percentage of anhydrouronic acid (a measure of pectin) varies but little. The cell wall structure is apparently constant with respect to this factor. The araban varies as widely as the marc content. There is a positive correlation between araban and sucrose ($r = 0.68$). Galactan percentage is low in the marc and was not considered sufficiently reliable for statistical analysis. No measurements are shown but a xylan or a complex of xylose and some other sugar was also noted in most of the pulps. This accounted for only 1 to 2 percent of the marc. Of the polysaccharides studied in this investigation only araban is indicated as a genetically variable characteristic. Present evidence indicates a positive correlation between sucrose content and araban; thus it may be impractical to decrease araban by breeding.

The marc content as determined indicates an average Sachs-Le Docte volume of about 176.6 ml., assuming no hydration of the pulp. Present indications are that the SLD volume may be higher than 176.6 ml. because of the presence of other optically active compounds and not because of unusual hydration of the marc.

The contribution of marc constituents to processing difficulties has never been systematically evaluated. Except for pectin, the physical and chemical properties of the polysaccharides are not sufficiently well known to predict their behavior during processing. Pectin is the best known and is a long chain molecule made up of galacturonide units (7). Some of the carboxyl groups are esterified with methyl alcohol while, in the case of beet pectin, some of the hydroxyl groups are esterified with acetic acid. There are present in pectin, in a more or less firm combination, arabinose, galactose, and rhamnose. It is also known that lime deesterifies and degrades pectin so that insoluble calcium pectate will form. The extent of the degradation is so dependent upon conditions that the findings of Finke (8) cannot be applied directly to American practice. Consequently, additional work in this field is needed.

The araban is apparently a low molecular weight compound (9) which will go through the defecators and into the thin juice. It may well be one of the gums reported to have deleterious effects on crystallization (10).

The galactan has not been studied.

Preliminary work by Goodban and Owens of this laboratory has shown that both araban and pectin are extracted much more effectively at pH 9 than at pH 6. The adverse effects on pressability of pulp, and sedimentation and filtration of first carbonation precipitate when alkaline battery supply water is used (11), may be the result of excessive extraction of these polysaccharides.

In summary, the marc content of the 15 different varieties of beets grown under the same conditions varied from 3 to 6 percent. There is a positive correlation between marc, araban, and sucrose contents of the inbred beets studied. Araban in the marc varies sufficiently among the varieties that it may be subject to control by the plant breeder.

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