Methods of Measuring Quality Losses in the Laboratory

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These losses encompass those physical and chemical changes during storage which lead to less efficient factory operations.

Among the physical changes we are concerned with are those caused by a loss of moisture, freezing, and attacks by microorganisms. These changes cause difficulty in slicing, decrease the mechanical strength of the cossettes, and increase the impurity load.

In addition to the standard methods of measuring the length of 100 grams of cossettes or the force required to rupture a standard disc of beet material, one might measure the nitrogen content of a sample of diffusion juice daily in order to see what increase is due to greater loss of cell content as the slicing quality of the beet deteriorates. Such a study is underway at Rocky Ford where we will determine total nitrogen by micro-Kjeldahl method using glass fiber discs on which 100 lambda of diffusion juice of known brix and apparent purity was dried daily at one factory.

This next year we are planning to incorporate a slicing test and, perhaps, a diffusion test in our work on stored beets. At present the slicing test is visualized as one where a standard core sample of whole beet would be placed in a holder on a load cell and a knife blade pushed through. The output of the cell would be integrated versus time. This should give a measure of power required to slice the beet tissue. The load cell and knife power output would be calibrated against a standard material such as a soft wax candle. One face of the cut beet tissue could be rested on a damp but tared piece of blotting paper for a standard length of time at a given temperature. The increase in weight after drying would be a measure of diffusion. The other face could be measured microscopically for number of ruptured cells per unit area. Of course the length of the core sample and area of the cut faces would be standardized or measured individually.

Non-destructive methods of testing such as the measurement of audio frequencies absorbed by beet tissue might offer a rapid and simple method of measuring beet quality as they have in studies of fruits and vegetables done at Beltsville, Maryland.

In order to measure chemical characteristics of sugarbeet varieties the experience at Rocky Ford has been that many many samples and replicates are necessary because of the innate variability of our varieties. Thus, over the years my predecessors P. C. Hanzas and R. F. Olson had developed efficient and simple methods to deal with these vast numbers of samples. When Dr. Carruthers brought forth his impurity value concept, work was soon begun to utilize it.

Essentially, this impurity value provides an approximation of those impurities which are in the beet at harvest and are diffused along with

sugar but are not removed in simple factory purification processes. These impurities then impede crystallization to such an extent that the loss of sugar in molasses accounts for about one-seventh of the sugar entering the factory even with high quality fresh beets. Determination of impurity value can be carried out on the same leaded filtrate used for direct polarization provided certain precautions are observed. Among these are: rather complete extraction, use of an extraction medium free of sodium and potassium, and use of a minimum of clarifying agent and low alkalinity. In our case the first is accomplished by use of frozen beet brei which has been stored at 0 F or lower and diffuses readily. The second and third conditions are met by using deionized distilled water dispensed by a specially calibrated Reyers balance and 1.9 grams, measured with a small dipper, of Hornes' dry lead per 26 grams of brei for clarification. The samples are stirred at 400-500 rpm for 2 minutes and filtered without delay. Portions of the filtrate are withdrawn for immediate determination of sodium and potassium on our Beckman DU spectrophotometer and for determination of amino nitrogen by the Carolan version of the Stanek-Pavlas method. The main portion of the filtrate is used for determination of direct polarization in the saccharimeter and a small vial of filtrate may be frozen and reserved for future use.

The calibration curves for the spectrophotometer are determined in the presence of varying quantities of sodium or potassium in addition to 100 ppm of calcium, magnesium, and the other, plus 2 percent sugar. PPM in the filtrate are converted to ppm on beet by the factor $\frac{1.0}{.13} = 7.7$

For determination of amino-N, 10 ml of leaded filtrate are added to a cuvette containing 1 ml of the Stanek-Pavlas reagent and the percent transmission is read at 575 nanometers after setting distilled water at 100% transmission. The reagent is prepared from 250 grams sodium acetate, and 10 grams copper nitrate dissolved and brought to 950 ml before adjustment to pH 6.2 cold with acetic acid. This is then brought to 1000 ml, allowed to stand at least overnight and filtered before use.

The calibration curve is developed versus a synthetic mixture of 9 amino acids in relative proportions as they exist in the average sugarbeet. PPM amino N in the filtrate is converted to PPM on beet by the factor $\frac{1.0}{.13}$ again.

Having the data for sodium, Na; potassium, K; and amino N, all in ppm on beet, it now becomes easy to calculate impurities (ppm on beet) as follows:

3.5 (ppm Na) + 2.5 (ppm K) + 9 (ppm N) equals ppm impurities.

A simple move of the decimal point four places gives percent.

If one assumes a 60 purity molasses, and consequently, $\frac{60}{100-60}$ to $\frac{60}{40}$ or 1 1/2 ratio of sugar to non-sugar in the molasses solids, it becomes possible for fresh beets, to estimate the amount of sugar in percent on beet which will likely end up in molasses simply by multiplying the percent

impurity value as determined from Na, K, amino N, by 1 1/2. If one then subtracts this new value from the initial sugar content of the sample and then divides by the initial sugar content one may calculate a relative extraction figure which would indicate the percent of original sugar ending up "in the bag" if molasses were the only loss. In order to make the data even more meaningful it is good practice to include an estimate of sugar loss to pulp, lime flume, and unaccountable loss along with the molasses loss when doing the above calculation.

Up to this point nothing has been said about the diffusable organic non-sucrose sugars which are present in small amounts in sound, freshly harvested, relatively mature beets, but increase a great deal during storage at the expense of sucrose.

The following may be easily determined by paper chromatography: levan, raffinose, kestose, glucose and fructose.

The method consists of hand squeezing a portion of the juice from the brei sample, transferring the juice to a vial and spotting 15 lambda, or .015 ml on the spot points of a previously prepared Whatman #4 chromatographic paper. The percent solids is determined on another portion of the sample using a refractometer. In our case we spot 15 lambda of standard solutions containing 1, 2, 4, 8, 12, 16, 20, 30, gamma, or micrograms, of each of the above sugars except kestose plus 10% sucrose to maintain the same spot size as in the unknowns. Thus we have 8 standard spots each of which contain 1 or 2, or 4, etc. micrograms of each of the above sugars. We use 18 1/4" X 22 1/2" sheets of paper with the spot line 5 1/2" from the end. Spots are placed 1" apart. A masonite board 5 1/2" wide and 22 1/2" long with notches at 1" intervals on the edge is handy for marking the 22 spot positions. The 15 lambda spots are dried immediately after spotting and are stable for months. Thus there is room for 7 known spots on either side of the 8 standards.

One and one-half inches of the paper above the spot line is folded back and stapled, and a glass rod slipped through. The chromatogram is developed descendingly in a stainless steel lined cabinet equipped with soaker sheets around the sides to maintain a saturated atmosphere and a blotter in the cover to prevent condensation and drippage on the chromatograms. Each cabinet holds 8 troughs and each trough has one paper having the 12-14 unknowns spotted on it. Since we have 6 cabinets our capacity is 576 unknowns per day. Each cabinet is surrounded with a 500 watt thermostatically controlled heater tape so as to maintain about 110 F during the run.

The solvent system used to develop the chromatograms is as follows: 70 parts by volume of isopropanol, 25 parts by volume of benzene, 10 parts by volume of n-butanol.

The mixture above is called solvent X. About 300 ml of distilled water are added to each 2 liters of solvent X before use to develop the chromatogram. With this mixture the sugars are well separated in 15 hours-overnight. The next morning the papers are removed, placed on racks, and dried in the well ventilated room.

To display reducing sugars, i.e. glucose and fructose, the dried paper is first drawn through a silver nitrate-acetone solution. This solution is composed of 10 grams silver nitrate dissolved in 30 ml distilled water and then mixed with 1 liter of acetone. After drying, the paper is drawn through an alkali solution face down and again dried. At this time the 1 gamma spot of fructose should be barely visible. The alkali solution is composed of 5 grams sodium hydroxide dissolved in 10 ml distilled water and added to 1 liter of isopropanol with mixing. Two phases develop. Best results are obtained by using the clear supernatant. If more sensitivity is desired, one can often redip in the silver nitrate solution and alkali solutions, drying between each. The sugars are displayed as dark brown spots against a light brown background with sucrose a rather prominent spot, fructose having moved the farthest, and glucose between the fructose and sucrose.

Sensitivity of the displayed spots and background areas to light may be stopped by finally drawing the paper through a 10% solution of sodium thiosulfate dissolved in 50% methyl or ethyl alcohol in distilled water. After drying the paper may then be kept indefinitely.

Papers on which levan, raffinose, kestose and fructose are to be determined are dried after removal from the cabinets then drawn through a solution consisting of 16 grams of high quality alpha naphthol dissolved in 1600 ml isopropanol and to which 250 ml of concentrated phosphoric acid has been added with mixing before use.

After drying again, these papers are subjected to a 90 C temperature for about two minutes in our ventilated oven in order to display the sugars. The violet colored spots locating the sugars last only for a few minutes before fading, therefore the papers should be evaluated immediately.

The sugars position themselves on the chromatograms as follows: Levan stays at the position of the original spot. Raffinose will be found just outside the original spot. Kestoses are found between raffinose and sucrose. Sucrose is a very prominent spot. Fructose has moved beyond the sucrose.

If levan is unavailable to put in the standards, one can place 15 lambda of various fructose concentrations at the position of the original standard spots on the spot line before the paper is dipped in the alpha naphthol solution and proceed as usual after drying the applied spots. Tests in our laboratory show that the color intensity developed by a given concentration of fructose is almost identical to that of levan at the same concentration.

Usually kestose is unavailable as a standard, therefore we grade the kestose spots versus the raffinose standards and divide by two.

It is very important that exactly 15 lambda always be spotted for both standards and unknown samples and that the size of the displayed spots be the same for both standards and unknowns. These two requirements are made easy by using a micropettor to do the spotting and 10% sucrose free of raffinose, etc. in the standards.

Evaluation of the sugars is carried out by visually comparing the color intensities and size of the unknown spots with that of the spots of known concentration.

Suppose: 1 gamma per 15 lambda raffinose is found in a juice sample of 15 brix having a density of 1.061.

Then: % raffinose on D.S. $= \frac{1 \times .0067}{.15 \times 1.061} = 0.042$ where D.S. stands for dissolved solids.

A set of specially prepared tables relating various quantities of gamma per 15 lambda to brix are available in our laboratory to speed this calculation.

If one wishes to have the concentration of raffinose in the juice spotted in the above example, he calculates 0.042 X .15 = .0063% on juice.

If one makes the assumption that the juice constitutes 95% of the beet, then he calculates .0063 X .95 = 0.0060% raffinose on beet.

If the concentration of a sugar is expected to exceed 30 gamma per 15 lambda or 30 grams per 15 liters, one should dilute the juice before spotting. A diluting pipet is useful for this.

At the conclusion of the analyses described thus far the following data are available:

1. Sugar by direct polarization.
2. Impurity value from sodium, potassium, amino N.
3. Levan, raffinose, kestose, glucose, fructose.

In addition, CO_2 respiration may be approximated by Stout's equation.

The data in 3, when calculated in percent on beet are useful for correcting the direct polarization and for estimating the build up of non-sucrose sugars during storage. Even an estimate of the original sucrose concentration can be attempted on a stored beet if one makes corrections for loss of CO_2 and moisture and assumes that all the non-sucrose sugars were formed at the expense of sucrose.

Work in our laboratory shows that approximately none of the glucose and half of the fructose lose their optical activity in a cold clarification of beet brei with basic lead acetate as compared to half the glucose and all the fructose in the case of a hot clarification of beet brei with basic lead acetate.

For a more accurate assay of the conditions of optical activity it is possible to spot 15 lambda of the leaded filtrate and determine, levan, raffinose, kestose, glucose, and fructose as described earlier. Since 1 gamma per 15 lambda of one-half NWS filtrate is almost exactly equal to .05% on beet, it is very simple to divide the gamma per 15 lambda determined in the filtrate by 20 in order to convert to percent on beet.

The following optical rotation factors are then applied to correct the sucrose percent determined optically:

1% levan on beet polarizes	-0.6% sucrose
1% raffinose on beet polarizes	+1.85% sucrose
1% kestose on beet polarizes	+0.4% sucrose
1% glucose on beet polarizes	+0.81% sucrose
1% fructose on beet polarizes	-1.42% sucrose

In order to get an accurate estimate of the non-sucrose sugars when analyzing stored beets, we have found it necessary to spot 6 papers for each sample of brei as follows:

2 papers at different press juice dilutions for glucose and fructose
2 papers at different press juice dilutions for the other sugars
1 paper for glucose and fructose in the leaded filtrate
1 paper for the other sugars in the leaded filtrate

All told we can do 200 samples per day determining direct polarization, impurity value and non-sucrose sugars utilizing 10 people.

The foregoing is meant to explain exactly how the chemical data reported by Mr. Swift and Dr. Watkins was obtained.

If we consider almost complete extraction of raffinose, kestose, glucose, and fructose from the beet during the factory diffusion process, and if we allow the supposition that they or their degradation products mostly pass through the purification process and into the molasses, then these non-sucrose sugars all contribute to the molasses brix and thus to viscosity at least, and probably all of them impede crystallization of sucrose, particularly in a factory with minimum raw pan and crystallizer capacity.

If one then further assumes that the ratio of sucrose to non-sucrose solids is 60 to 40 in the molasses then he can state that the presence of 1 pound of non-sucrose in the molasses carries 1 1/2 pounds of sucrose with it.

To check out this idea a series of tests were performed on the 24 hourly cossette samples at a factory which were frozen and shipped to Rocky Ford. The data are tabulated in Table 1.

Thin juice purity test (phosphated) was performed in a slightly different manner than normally: for these tests we used 2.5% CaO on beets and the samples were held at 60 C for 10 minutes before the first filtration. The purpose of changes was to imitate actual factory operations more closely.

Note the close correlation between determined thin juice purity and thin juice purity calculated from direct pol., impurity value, and non-sucrose sugars.

If one accepts the idea that thin juice purity test does in fact imitate factory operations in the laboratory, then it follows that we are now analyzing most of those factors which cause molasses formation and consequently can estimate molasses formation from the analysis of individual brei samples.

Table 1. Hourly cossette samples from beets stored 120 days at factory M. Samples were frozen at the factory and analyzed at Rocky Ford.

	Cossettes	ppm on Beet				Percent on Beet							Thin Jc. Purity		
Sample	Dir. Pol.	Na	K	Amino N	Imp. Value	Raff.	Kes.	Gluc.	Fruc.	Levan	Corr. Sugar	Tot. Imp.	Lab.	Calc.	Rel. Ext.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
1	14.8	550	1420	354	8661	.18	.04	.16	.10	.01	14.4	1.35	93.6	91.4	80
2	14.8	520	1850	361	9694	.16	.03	.13	.06	.01	14.4	1.35	92.2	91.4	80
3	12.7	640	1620	371	9629	.13	.05	.26	.16	.10	12.3	1.70	85.4	87.8	72
4	12.5	580	1920	462	10988	.18	.08	.33	.18	.10	12.0	1.87	86.4	86.5	69
5	13.2	520	1770	450	10295	.16	.04	.14	.16	.06	12.9	1.53	90.5	89.4	76
6	13.2	550	1920	454	10811	.18	.05	.18	.14	.08	12.8	1.63	87.9	88.7	74
7	13.4	450	1770	354	9186	.16	.04	.18	.16	.05	13.1	1.46	90.0	90.0	77
8	13.6	550	1920	384	10151	.16	.03	.18	.10	.00	13.2	1.49	90.7	89.8	76
9	13.1	490	1850	442	10318	.14	.04	.21	.12	.03	12.7	1.54	89.3	89.2	75
10	15.2	250	1920	331	8654	.13	.01	.13	.09	.02	14.9	1.23	93.8	92.4	82
11	13.6	520	1690	425	9870	.13	.04	.16	.10	.00	13.3	1.42	90.0	90.4	78
12	14.6	610	1620	366	9479	.16	.01	.13	.08	.01	14.2	1.33	92.7	91.4	79
13	14.6	420	1540	384	8776	.16	.04	.21	.08	.01	14.2	1.37	91.9	91.2	79
14	11.6	480	1690	420	9685	.21	.10	.52	.39	.05	11.0	2.19	82.9	83.4	61
15	13.5	480	2000	458	10802	.18	.05	.26	.27	.02	13.1	1.84	88.7	87.7	72
16	11.8	490	1620	442	9743	.13	.05	.39	.35	.10	11.5	1.89	86.1	85.9	69
17	15.1	400	1540	366	8544	.13	.04	.13	.06	.01	14.8	1.21	92.7	92.4	82
18	13.1	670	2000	431	11224	.13	.05	.23	.16	.10	12.8	1.69	92.3	88.3	74
19	15.5	330	1770	361	8829	.13	.05	.18	.13	.01	15.2	1.37	91.4	91.7	81
20	14.4	490	2230	442	11268	.13	.04	.16	.10	.00	14.1	1.60	91.4	89.8	77
21	14.5	450	1770	342	9078	.16	.03	.16	.10	.00	14.1	1.36	91.3	91.2	79
22	14.6	490	2490	431	11819	.13	.03	.16	.15	.00	14.3	1.65	91.9	89.6	77
Avg.	13.8	497	1814	401	9888	.15	.04	.21	.15	.03	13.4	1.55	90.1	89.5	76

(1) Two Samples Missing.

(2) Blended 5 min. cold with Horne's Lead.

(5) Stanek-Pavlas

(6) 3.5 (3) + 2.5 (4) + 9 (5)

(12) (2) -[+1.85 (7) +.4 (8) +.8 (9) -.71 (10)]

(13) (7) +(8) +(9) +(10) +[(6) ÷ 10000]

(15) [100 (12)] ÷ [(12) +(13)]

(16) [(12) -1.5 (13) -*.6] 100 ÷ (2)

*.6 = Allowance for factory losses except molasses

Using the outlined methods, one can separate the chemical loss of "sugar in the bag" during sugarbeet storage into the following component parts:

1. That apparent loss due to false polarization, i.e. due to what appeared to be sucrose but in fact is not sucrose at the time of processing.

2. CO_2 respiration during storage via Stout's equation.

3. Metabolism at the expense of sucrose in forming levan, raffinose, kestose, glucose, fructose and perhaps dextran, etc.

4. Increased loss of sugar to molasses due to buildup of the non-sucrose sugars.

5. Increased loss of sugar due to spills caused by the presence of levan and dextran which lead to many factory difficulties, especially filtration.

In considering what may be done about these losses I believe that in most cases we need to get control of enzyme systems. But in considering some of these losses at least, I believe we need to separate the enzyme systems inherent in the beet from enzyme systems arising from invasion by microorganisms if genetic progress is to be made. The fact that invasion of beet tissues by microorganisms is often accompanied by formation of levan is a point in favor of paper chromatography as a means of analysis. Thus if one wishes to separate glucose and fructose production in the natural metabolism cycle from other causes he could easily throw out those chromatograms showing levan present. Dextran is also an indicator and I think the described paper chromatographic system could be modified for its determination by using glass fiber paper and sulfuric acid.

Possibly, on freezing and thawing the sugarbeet cells lose control of their enzymes. This may account for the observation that the beets look good when in fact they process with great difficulty.

More modern laboratory analytical methods include that of the gas liquid chromatograph (GLC) which gives data on sucrose free of the problems of optical activity of other components of the solution. It will be interesting to see how our determinations of sucrose by GLC match those inferred from direct polarization and paper chromatographic data. This instrument may also be used to determine more accurately what happens to glucose and fructose in the factory.

Nitrate concentration is another factor which may affect storageability of the sugarbeet. We have had very good results using the orion nitrate electrode with a double junction reference electrode in a flow through cell placed in the flow path of the leaded filtrate after the sugar reading is taken. We use 1% potassium nitrate in the outer chamber of the reference electrode and find that this system reads 80% of the nitrate present when concentration in the leaded filtrate is compared with the concentration in a comparable silver sulfate extract.

Some preliminary work has been done on pH of filtered 1/2 NWS distilled water extracts of frozen beet brei produced from beets treated with a wide range of fertilizers. We have been disappointed at the narrow range of pH differences of .3 to .4 among the extracts as well as among the mating press juice samples.

Specific conductance too may be a measure of storageability. We have some work underway in this area and hope to be able to read significant differences in leaded filtrates using apparatus now being developed.

On the supposition that concentration of reducing sugars are indicators of beet quality a very rapid and simple test has been devised by a modification of the TTC test described by Oldfield, Dutton, and Teague in the International Sugar Journal, 1971, 73, P. 68.

The modified test is carried out on a white glazed porcelain plate heated on a boiling water bath and on which a few drops of known concentrations of glucose-fructose are placed in a streak alongside a streak of juice derived from the beets in question. A few drops of the TTC reagent are dripped on each of the two streaks and the color development noted. The reagent is the same as described by Oldfield and the test is complete in 20-30 seconds. Comparison of the developed color by this test permits one to separate beets into three general classes. Those having less than .2% reducing sugars, those having .2 to about .7% and those having more than .7%. Application of this test to the hourly cossette sample in a factory laboratory for a campaign could define its usefulness as a measure of quality.