Effects of Sugarbeet Sample Preparation and Handling on Sucrose, Nonsucroses and Purity Analyses *

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Sucrose recovery from sugarbeet (*Beta vulgaris* L.) is not only a function of sucrose concentration in the roots but also of the proportion of sucrose which can be crystallized from purified beet juice. The proportion of recoverable sucrose is affected by the quantity of soluble nonsucrose compounds in the roots relative to sucrose, but also it may be affected by a particular factory process. Several of the soluble nonsucrose compounds present in beets affect the solubility and rate of crystallization of sucrose. Hence, these compounds and other quality factors must be measurable or assessable in order to evaluate the quality of the beets and various processing juices.

Quality assessment of the beets is a necessity in breeding, agronomic, storage and other research. Sampling and assessment for quality is done on a large scale in sugarbeet research, but not necessarily in a standard manner. Certain quality analyses such as laboratory thin juice purity, as developed by Brown and Serro (1) and modified by Carruthers and Oldfield (2), are relatively standard but not extensively used because of high cost. However, sample preparation and handling for purity and other assessments are not standard. These techniques are largely dictated by local facilities, number of samples, and experience.

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Frequently, requirements of experiments necessitate preservation, transport, storage, and delayed analyses of beet juices or brei (finely subdivided root tissue) samples. Little published information exists to guide scientists in sample preparation and handling for these circumstances.

We have conducted a series of integrated experiments over several years to compare a number of standard and practical methods of sugarbeet sample preparation and preservation for sucrose and quality analyses from beets or different genotypes grown under different levels of nitrogen fertility. This report summarizes these results and presents comparisons of various sample extraction and treatment effects on measurements of polarimetric and gas chromatographic sucrose, glucose, purity, and the important nonsucrose purity components.

MATERIALS AND METHODS

All sugarbeet samples used in this study were from irrigated field experiments at Fort Collins, Colorado, planted in April and harvested in early October in several years. Polarimetric sucrose (suc) was measured in lead subacetate clarified solutions standard in the beet sugar industry (modified Sachs-Le Docte method). Gas chromatograph sucrose (GC suc) and glucose (gluc) were measured as described by Maag and Sisler (6). Quantitative measurements were made as described by Maag et al. (5) for ash by conductance, for nitrate (NO_3) by nitrate ion electrode, for chloride (Cl) by titration with silver, for sodium (Na) and potassium (K) by flame photometry, for amino-nitrogen (AMN) by ninhydrin reaction, and for total nitrogen (tot N) by a modified Kjeldahl technique (4). These determinations were all reported in mg/100 g sucrose in the respective extract or juice.

The necessity of transporting and delaying sample analyses of sugarbeet juices sometimes requires the use of a preservative to prevent microbiological activity. We conducted an experiment to assess the effects on juice quality characters of phenylmercuric acetate (PMA) at 50 ppm as an extract preservative. The 165 samples in this experiment were purposely heterogeneous. Equal parts (w/w) of sugarbeet

brei and boiling distilled water were blended 5 minutes and vacuum filtered. The samples were split (one of each pair receiving 50 ppm PMA) and analyzed for suc, refractive dry substance (RDS), percent purity (pur), ash, NO_3, Cl, Na, K, AMN, and tot N. The samples were analyzed immediately for pur, suc, and RDS, and refrigerated during the 2 days until the other analyses were completed. We did not study the efficacy of PMA as a preservative.

Since sugarbeet brei samples sometimes need to be collected, then frozen for later sucrose analysis, we conducted experiments in 4 years comparing the quantities of sucrose in fresh and frozen brei from beets grown at different nitrogen (N) fertility levels. The experiments were grown at low optimum, or excess N fertility. Excess N was applied as a treatment to simulate the high N levels which sometimes occur in commercial production. Each brei sample was thoroughly mixed and split, one-half analyzed for sucrose immediately, and the other half frozen immediately and stored frozen at -30°C for about 1 month.

Another aspect of this study involved four experiments comparing different methods of juice extraction and subsequent handling. The following extraction methods were used for these experiments:

1:1 ext. = equal parts (w/w) sugarbeet brei and distilled water, blended 3 min., vacuum filtered through Whatman No. 1 filter paper, and analyzed immediately.

1:1 ext. froz. = extracted as above, stored frozen at -30°C.

1:1 froz. brei ext. = brei stored frozen at -30°C, extracted as above.

Standard lab thin juice = pressed juice, limed, and stored frozen at -30°C.

All frozen samples were thawed at 20°C. The samples in these experiments were analyzed for the same characters as the PMA experiment. The beet samples were from four split plot and randomized complete block field experiments with four to ten replications.

Since the filtrate from sucrose analysis is always available, it is a practical sample from which to make other quality determinations. We conducted one experiment in which quantities of Na, K, and AMN were measured in 100 samples of fresh sucrose filtrate and identical subsamples stored 24hrs, or stored frozen and thawed at 4 °C, 37 °C, or by microwave. In another experiment, sucrose filtrate from fresh brei and frozen brei was compared with a frozen brei extract for concentration of Na, K, and AMN. The data from this experiment are reported in mg/100 ml, since each treatment was a subsample and conversion to mg/100 g sucrose would have confounded nonsucrose measure with any treatment effect on sucrose. All other nonsucrose data in this study are reported in mg/100 g sucrose.

To assess storage methods of samples for purity analysis, we compared thin juice purities of limed pressed juice, (1) immediately after collection, (2) after 30 days of storage at -30 °C and then thawed at 20 °C, (3) from brei frozen for 30 days at -30 °C and then microwave thawed, and (4) after 14 days of storage at 4 °C. Thoroughly mixed brei samples from 100 plots of beets in a randomized complete block experiment were divided into four subsamples for these treatments. Thin juice was prepared using the modified method of Carruthers and Oldfield (2).

A comparison was made of sucrose determinations by polarimeter and gas chromatograph in 10 cultivars in one randomized complete block field experiment with 10 replications. Glucose content was also determined in this experiment. All three measures were in percent of root fresh weight.

RESULTS AND DISCUSSION

A comparison of sugarbeet brei extracts with and without 50 ppm PMA is shown in Table 1. From these 165 paired samples the only PMA effects were significantly lower ash and AMN contents. All other quality and nonsucrose characters were the same for the control and PMA treated samples. The differences for ash and AMN, although significant, were not sufficiently large to be of practical importance.

It appears that PMA used as a sugarbeet juice preservative does not affect sucrose or quality determinations.

Table 1. Quality and nonsucrose characters in a 1:1 sugarbeet brei extract with and without 50 ppm phenylmercuric acetate (PMA); 165 paired samples.

	1:1 Extract				mg/100 g sucrose						
Trmt.	Thin juice pur	Suc	RDS	Ext pur	Ash	NO_3	Cl	NA	K	AMN	Tot N
No PMA	90.5	6.9	8.42	81.1	4967	1560	1800	1067	1200	330	1450
PMA	90.4	6.9	8.39	81.0	4713*	1547	1733	1070	1200	317*	1490

* Significantly different (5%) from extracts with no PMA, using a paired t test.

A comparison of the sucrose content of paired samples of fresh brei and frozen brei from beets grown at low, optimum, or excess N fertility showed no significant differences in the four experiments shown in Table 2. The sucrose differences between low N and excess N were all significant, but there were no differences between fresh and frozen brei sucrose within these N treatments. Cormany (3) reported no differences between fresh and frozen brei sucrose in a test of 25 varieties. Hence, it appears that freezing the brei has no effect on the measurement of sucrose. This allows considerable flexibility in scheduling and transporting brei samples for sucrose analysis.

Table 2. Comparison of pol sucrose content (%) determined from fresh brei and frozen brei.

	Experiment					
	1		2	3		4
	Opt. N.	Low N	Excess N	Opt. N.	Low N	Excess N
Fresh brei	13.6	14.3	13.7	16.7	18.4	16.4
Frozen Brei	13.8	14.2	13.6	17.1	18.5	16.6
No. samples/mean	55	100	100	48	14	14

The various methods of juice extraction examined for quality and for quantity of sucrose compounds are shown in Table 3. Of principal interest was the comparison of thin juice

purities of the extracts. All these thin juice purities were determined on the extracts by the method of Carruthers and Oldfield (2), with the appropriate quantity of lime being added just prior to purity analyses. The standard laboratory thin juice purities were determined on subsamples of pressed limed juice.

In the first experiment a comparison was made between 1:1 extract and 1:1 frozen brei extract, which was not different for thin juice purity, 90.1 and 90.0, respectively.

Refractive dry substance (RDS) and extract purity were determined in these same extracts. Their RDS's and extract purities were not different at 8.48 and 8.42, and 83.6 and 84.3, respectively.

In the second experiment the same two extracts were made and, additionally, one extract was stored frozen. The 1:1 extract, the 1:1 extract stored frozen, and the 1:1 frozen brei extract did not differ significantly with thin juice purities of 93.2, 93.4, and 93.0, respectively. However, standard laboratory thin juice purity of 95.8% was significantly different from the first three extraction methods. The leaded sucrose filtrate was included in the second and the third experiments only to provide some comparative data for the nonsucrose characteristics in the various extracts.

In the third experiment only a 1:1 frozen brei extract was tested. This extraction method was both practical and convenient and, thus, of greatest interest. Again, in this experiment the thin juice purity of this extract was significantly lower than the standard laboratory thin juice purity, 95.7 and 96.3, respectively. However, in a fourth experiment there was no difference between the purities of the 1:1 frozen brei extract and the standard laboratory thin juice, 94.9 and 94.7, respectively. Therefore, in 2 years out of 3 the thin juice purity of 1:1 frozen brei extract was significantly lower than that of standard laboratory thin juice prepared from limed pressed juice. Apparently there is a treatment by year interaction, implying

that purities of different extracts should not be used over years for comparative purposes. However, in these experiments the associated variances were not different. Therefore, consistent use of one or the other extract for thin juice purity determination should be equally acceptable for research purposes.

GC sucrose was measured only in the extracts of the second experiment (Table 3). The 1:1 frozen brei extract had significantly higher pol and GC sucrose than the other extracts. However, this was not reflected in the thin juice purity because of a commensurately higher RDS.

The nonsucrose compounds in the various extracts (Table 3) did not differ in the first experiment. Among the extracts in the second experiment, those differences that were significant were not sufficiently so to be of practical concern. A difference that was of practical consideration was in the quantities of nonsucrose constituents in the leaded sucrose filtrate compared to the standard lab thin juice in the second experiment. The differences for these nonsucrose constituents could have arisen through differential extraction of nonsucroses into the different extracts, component losses or transformation related to the chemical clarification procedure, or degradation of sucrose in the extraction process. The low quantity of total N in the lab thin juice is explained by the precipitation of many nitrogen-containing compounds, particularly proteins, in the laboratory thin juice process.

Since sucrose filtrate is extensively used for beet quality assessment, different methods of storing this filtrate were compared (Table 4). After 24 hours of refrigeration Na and AMN were significantly lower than in the samples freshly analyzed; whereas, K was unaffected by this treatment. On the other hand, the concentration of K was significantly altered by freezing the sample no matter what thawing procedure was employed; whereas, Na and AMN concentrations in samples that had been frozen were not significantly different from those of freshly analyzed samples. The differences among these treatments, although significant, were not large.

Table 3. Quality and chemical characters of sugarbeet extracts and juices over 4 years.

Experiment and treatment	T.J. % pur	% in Extract Pol suc	% in Extract GC suc	mg/100 g sucrose Ash	NO_3	Cl	Na	K	AMN	Tot N
Exp. 1										
1:1 ext.	90.1	7.2		4778	1480	174	1028	1119	297	1353
1:1 froz. brei ext.	90.0	7.2		4721	1494	169	1066	1158	303	1424
Exp. 2										
1:1 ext.	93.2*	7.1	7.0	3283	154	97	273*	722	120*	647*
1:1 ext. froz.	93.4*	7.0	6.8	3276	151	95	267	722	112*	608*
1:1 froz. brei ext.	93.0*	7.5†	7.5†	3244	163	101	292	709	108*	591*
std. lab thin juice	95.8	11.6‡		3228	167	104	354	710	86	278
leaded suc. filt		1.8‡			120 *	47*	496*	884*	96*	807*
Exp. 3										
1:1 froz. brei ext.	95.7*	8.6		2155	11	40	49	667	24	519
std. lab thin juice	96.3	12.5‡								
leaded suc. filt.		2.1‡			20†	23†	71†	772†	65†	318†
Exp. 4										
1:1 froz. brei ext.	94.9	8.9								
std. lab thin juice	94.7									

*Indicates significant difference (5%) from the standard lab thin juice within each year.

†Indicates significant difference (5%) from the first treatment within each experiment.

‡Not comparable statistically with the other treatments within each experiment due to dilution difference.

Table 4. Means of freshly analyzed sucrose filtrate samples compared with identical subsamples handled by different procedures.

Lead-clarified sucrose filtrate	Na	K	AMN
	---------mg/100 ml----------		
Freshly analyzed	7.82	21.71	3.56
Fresh, after 24 h at 4°C	7.66**	21.85	3.37**
Frozen, thawed 18 h at 4°C	7.74	22.88**	3.54
Frozen, thawed 37° water	7.77	22.73**	3.52
Frozen, microwave thawed	7.87	22.01**	3.48

** Significant difference from the freshly analyzed samples by paired t-test ($p = 0.01$)

In Table 5 data from another experiment show that the Na and K concentrations in fresh brei sucrose filtrate were higher than in frozen brei filtrate which was then frozen and microwave-thawed, but the AMN concentrations were not different. The Na and K in the 1:1 extract of frozen brei were not different than either of the two filtrates. Only AMN in this 1:1 extract was significantly higher than both filtrates. Hence, the concentrations of Na, K, and AMN in relation to sucrose were not consistently similar in sucrose filtrates, which, in turn, were not consistently different than the 1:1 extract of frozen brei. The correlations of these characters across the filtrates and extract also are shown in Table 5. These correlations are all positive, similarly high, and significantly greater than zero. It appears that concentrations of Na, K, and AMN measured in filtrates or beet extracts may be different quantitatively, but are proportionately similar. Hence, the type juice used may be immaterial provided the same juice and procedure are used consistently.

Table 5. Means and correlations (r) of filtrate and extract samples.

Extract	Na	k	AMN
	--------mg/100 g sucrose------		
1 Fresh brei sucrose filtrate	404 a*	1102 a*	180 B*
2 Suc filtrate from 1:1 extract of frozen brei; filtrate frozen & microwave thawed	342 b	953 b	195 b
3 1:1 extract of frozen brei	363 ab	938 b	229 a

Table 5. Continued.

	Correlations					
	Na		K		AMN	
Extract	2	3	2	3	2	3
1	0.96	0.97	0.92	0.93	0.98	0.98
2		0.96		0.94		0.98

* Means within columns followed by the same letter are not significantly different (5%).

Our interest in these extracts and filtrates as samples for determination of quality and quality related compounds is in part governed by practicality. Obviously, any juice requiring preparation and immediate analysis is impractical for a large number of samples unless a rapid, fully automated analysis of the components of interest were in use. Among the extracts tested, the most practical would probably be immediate extraction with the extract being stored frozen for later analyses, or frozen brei being later thawed, extracted, and analyzed. In the latter procedure, immediate freezing of the brei sample is important. From the standpoint of extract preparation alone, the sucrose filtrate would be an ideal juice with which to work as it is always available from the sucrose analyses. Its possible drawbacks are in the analytical complications and possible compositional alterations introduced by the lead or aluminum compounds (7) added in the sample defecation process. In our experience the 1:1 frozen brei extract is second in practicality to sucrose filtrate, followed by the 1:1 extract stored frozen, and the 1:1 extract analyzed immediately. Each of these can be prepared quickly and in large numbers. From a practical standpoint, we feel that the 1:1 frozen brei extract could provide an accurate assessment of the composition of beets. At the same time, this extract is one that can be practically utilized in all labs, even those with limited resources. It appears that useful quality component analyses can be made on any of several sample types with the important qualification that, within a lab, extract preparation and the subsequent method of handling samples must be

standardized. The choice of extract type for quality measurements (at least of Na, K, and AMN) may be one of convenience, but data from different extract types should not be directly compared numerically.

Thin juice purities of samples handled in four different manners are shown in Table 6. All four treatments are significantly different. The pressed juice which was analyzed immediately had the highest purity, 93.7%. It seems likely that this should be the most accurate measure since there was little opportunity for sample deterioration. The lower purity of limed juice stored frozen, 93.4%, is difficult to explain because there were only a few minutes between sampling the beets and adding lime to the juice, after which the juice samples were frozen and then thawed before analysis. Even though there may have been several hours involved in the freezing and thawing process, the pH of about 12 should have been an environment too hostile for significant microbial activity, although some enzymatic activity may have taken place. The still lower purity of juice from frozen brei, 92.8%, could have been caused by some sucrose degradation in the brei before freezing and during thawing. Limed juice stored 14 days at 4° C had the lowest purity, 92.1%.

Table 6. Thin juice purity of four pressed juice treatments.

Treatment	Thin juice purity (%)	RDS	Pol
Limed juice, analyzed immediately	93.7 a*	11.54	43.42
Limed juice, stored frozen	93.4 b	11.55	43.28
Limed juice from frozen brei	92.8 c	10.46	38.82
Limed juice, stored 14 days at 4°C	92.1 d	12.65	46.98

*Means with the same letter are not significantly different (5%).

The components of purity in Table 6, RDS and polarization, indicate that the sucrose content declined in limed juice stored frozen and in juice from frozen brei, while RDS (soluble solids) decreased in the latter juice. In the limed juice stored 14 days at 4° C the RDS apparently increased, but the soluble solids consisted of proportionately

less sucrose so that the resulting purity was significantly lower than all other juices. This increased RDS apparently resulted from solubilization of insoluble nonsucrose compounds during the 14-day storage period.

The comparison of pol and GC sucrose determinations in 10 cultivars is shown in Table 7. No comparison within cultivars was significantly different (25 samples per mean), and the means across cultivars were identical, 14.4%. Hence, across these cultivars with wide ranging sucrose values, the pol and GC sucrose determinations were the same.

Table 7. Polarimetric and gas chromatographic sucrose and glucose content of 10 cultivars in percent of fresh weight.

Cultivar	Sucrose (%) Pol	GC	Glucose(%)
US H9B	14.9 cd*	14.9 c*	0.14 c*
US H20	14.3 d	14.0 d	0.23 b
HH 10	15.6 abc	15.6 bc	0.15 c
GW 359	15.9 ab	15.8 bc	0.20 bc
GW Mono Hy	16.1 ab	16.3 ab	0.15 c
Exp. hyb 1	16.4 a	16.8 a	0.16 c
Exp. hyb 2	15.3 bc	15.5 bc	0.17 c
Exp. hyb 3	15.9 ab	15.9 b	0.16 c
Sugar X fodder beet, F_1	11.9 e	11.8 e	0.18 b
Fodder Beet	7.6 f	7.5 f	0.43 a
Mean	14.4	14.4	0.20

*Means within columns followed by the same letter are not significantly different (5%).

The glucose means in Table 7 show that relatively small quantities of glucose are present in the eight sugarbeet cultivars, averaging 0.17% on a fresh weight basis, compared to 15.6% sucrose. The fodder beet contains considerably more glucose (2.5 times that in sugarbeets) and less sucrose (about half that in sugarbeets). The sugarbeet X fodder beet hybrid was essentially intermediate for sucrose, but similar to sugarbeet in glucose content.

SUMMARY

Several experiments were conducted to compare methods of sugarbeet *(Beta vulgaris* L.) sample preparation and preservation for sucrose and quality analyses. Samples treated with the preservative phenylmercuric acetate (PMA)

were not different from controls for sucrose, refractive dry substance, raw juice purity, and thin juice purity. Among seven nonsucrose components of purity, amino nitrogen and conductivity ash were present in significantly lower quantity in the PMA treated samples.

In separate experiments comparing fresh and frozen brei, sucrose contents of the two brei treatments were the same in beets grown at low, optimum, and excess nitrogen fertility.

Three methods of juice extraction were found to have similar thin juice purities. However, in two of three experiments the extracts had significantly lower thin juice purity than standard limed pressed juice stored frozen. Differences among seven nonsucroses in the extracts were significant in some cases but not practically important. Each of the extracts studied should provide a reliable purity sample. Practically, the 1:1 frozen brei extract should provide an alternative method which permits an accurate assessment of the constitution and composition of beets. However, data from different extract types should not be compared numerically.

The Na, K, and AMN contents of lead-clarified sucrose filtrate and brei extract were significantly affected by some filtrate treatments. However, high correlations among the different filtrate treatments indicated that the juice treatment or type may be immaterial provided the same juice and procedure were used consistently.

Three methods of storing brei or limed juice samples all exhibited lower thin juice purity than immediately analyzed juices.

Glucose in 10 diverse cultivars ranged from 0.14% to 0.43% of fresh root weight. Sucrose measurements by polarization and gas chromatography were not different in any of these 10 cultivars.

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