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The appropriate method for determining of sugar content in sugar beet produced under drought, salinity and normal conditions

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ABSTRACT

In polarimetric method of sugar content determination, the extract derived from 26 g of normal sugar beet brei is considered to be 23 ml which is added to 177 ml clear solution to make a total volume of 200 ml . The increased dry matter and sugar beet pulp under any type of stress and flaccid may decrease the volume of 26 g sugar beet extract from 23 ml. This study was conducted to determine the sugar content of the sugar beet produced under drought and saline conditions and also flaccid roots in the field after harvest. The studied roots were produced under normal, drought, semi-saline and saline conditions in two regions of Karaj and Isfahan in 2010. After harvest, roots were exposed to flaccidity and were tested independently as a 3×3 factorial experiment based on randomized complete block design in twelve replications. Root flaccidity factor (A) included three levels: a1- fresh root produced at optimum condition with 76±1% moisture content, a2- flaccid root in about 70±1% moisture content, a3- completely flaccid root with 65±1% moisture content, and extraction method factor (B) included three levels: b1- cold digestion extraction method, b2- French method based on extract volume, and b3- hot digestion method. Sugar percentage, dry matter percentage, pulp percentage (marc), brix and electrical conductivity of all samples were measured. The results showed that root flaccidity increased the root dry matter from 24 to 32% and the pulp from 4.7 to 7.1%. No significant difference was found between conventional extraction with French method and hot digestion method for normal roots but differences in average saline stress ($P<0.05$), drought and saline stress ($p<0.01$) were significant. Overall, no significant difference was observed between French extraction and hot digestion methods in all conditions. No significant difference was found between conventional and French methods in the range of 24 to 27% dry matter ($p<0.01$). Based on the results of this study and the simplicity of cold digestion method, it is recommended to use cold digestion method for the roots with dry matter up to 27% and French method for the roots with dry matter more than 27%.

Keywords: cold digestion, drought stress, extraction, flaccid, hot digestion, polarimetry, saline stress, sugar beet

INTRODUCTION

In sugar content determination of sugar beet by using polarimetric method, sugar extraction from sugar beet brei is called digestion. In this regard, correct dilution (addition of mixed clear juice) and extraction (digestion) have a high importance to make the extract of digest be filtered easily. Several methods were used for sugar beet brei digestion and are still developing. Kunz (2004) has introduced some methods for this purpose. Sample selection in sugar beet brei for sucrose content determination is carried out based on the increasing method of Sachs (Sachs and Le-Docte 1970) so that 26 g of the root brei is weighed and mixed with 177 cm^3 alkaline lead acetate at 20 ^oC [177 cm^3 volume selection is based on the assumption that 26 g sugar beet pulp has 23 cm^3 volume and according to ICUMSA instruction, the dry matter of 26 g sugar beet pulp with 77% moisture equals to 5.9 g (Kunz 2004)]. By balancing the concentration between tested sample and alkaline lead acetate mixture, a mixture of 200 cm^3 volume will be obtained (Sheikholaslami 1997). The accuracy of the method depends on the extract vol-

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ume and the brei marc content of sugar beet. The results of the research conducted on this subject showed that the extract volume of 26 g sugar beet brei with respect to marc percentage was between 20.7-23 ml (Van der Por et al. 1998). Broad analysis of sugar beet brei in Europe also shows that in some cases the volume of the extract was 20.7-21.8 which was practically considered 23 ml. This deficiency can be intensified when an equal volume of clear mixture is added to all brei samples. Fluctuations in volume of the brei extract can be influenced by the cultivar, soil type, and climate conditions in sugar beet production area (Van der Por et al. 1998). Moisture content reduction in the roots of sugar beet may be caused by drought stress, low irrigation during the growing season, or long-term exposure of the harvested roots to the air (root flaccidity). Moisture content reduction in root may cause a reduction in extract volume and an increase in brei marc percentage. In this regard, Hrichmuller and Krocher (1968) determined the solution volume of acetate on the brei samples of sugar beet by conducting 90 experiments using isotopic dilution technique. A series of comparative tests were conducted in October, November, and January for sugar beets kept in cold storage condition and under the sun. Sugar beets storage under the sunlight resulted in water loss, increase in average dry matter by 26.9, 31.4, and 31.3% along with sugar increase by 20.4, 23.4, and 24%, and also increase in marc by 5.2, 5.5, and 6.3%. On the other hand, studies showed that in low irrigation situation, the root yield of sugar beet decreased (Parvizi Almani et al. 1997) but the brei percentage increased (Mohammadian et al. 2011). Studies also illustrated that salt increase caused an increase in the percentage of soluble solid materials in the roots and change in chemical composition of the root compared with a root grown in non-stress condition (AbdelMawly and Zanouny 2004). Jahadakbar et al. (2005) studied the effect of salinity in irrigation water (4, 8, and 12 ds/m) on the quality and quantity of sugar beet and showed that salt increase resulted in the reduction of root yield and by inhibiting the plant growth, plants were remained in small size. To reduce the measurement error of the brei samples extracted from sugar beet which didn’t reach to 200 ml volume with 177 ml clear solvent, the French Sugar Industry Research Institute has introduced an equation by which the clear mixture volume for the root samples with different moisture percentages can be corrected. In this method, the extract volume of sugar beet brei can be calculated by equation 1 (Van der Pol et al. 1998):

$$V_j = \frac{SB \times (100 - MC)}{100 \times dB} \tag{1}$$

where Vj is the volume of sugar beet extract (26 g) based on ml, SB is the sugar beet brie weight (26 g) based on g, MC is the pulp weight based on g, and dB is the extract density of sugar beet brei based on g/cm^3. In this equation, by the pulp weight difference from 100 g, sugar beet brei soluble solid weight of 100 g brei based on the gram is calculated, and by dividing this value to the extract density, it is converted to volume based on ml, and by multiplying it by 0.26, the extract volume of 26 g pulp is calculated. A two-stage digestion of the sugar beet pulp (hot digestion) is also one of the methods to reduce the error associated with the measurement of cold digestion method. In this method, extraction and sugar content measurement is done in two stages by which the total sugar percentage is calculated from the sum of both measurements. This technique reduces the errors caused by extract and marc volume (Parker 1970). The objective of this study was to determine the most appropriate extraction method by comparing the existing methods in respect to dry matter, marc, extract density, and extract volume obtained from 26 g sugar beet pulp sample, which was produced under normal, drought and saline stress conditions in the field, and also root flaccid after harvest. In addition, sugar content measured by both conventional and corrected methods was compared.

MATERIALS AND METHODS

In this study, the sugar beet roots which were transferred to the Sugar Technology Laboratory were divided into five groups based on the origin:

1. Roots from control samples collected from Karaj region without salinity and drought restriction during the growing season,
2. Roots collected from drought stress condition (deficit-irrigation in the field as a circuit after plant establishment) in Karaj region,
3. Roots of control samples from Isfahan region without salinity and drought restriction during the growing season,
4. Roots produced in Isfahan region with water quality about 8 ds/m (medium salinity),
5. Roots produced with water quality about 12 ds/m (saline).

Study was carried out in a 3×3factorial experiment based on randomized complete block design in twelve replications for five types of sugar beet with different qualities in laboratory condition.

Flaccidity percentage (A) was measured at 3 levels including:

a1- Fresh root produced at optimum condition with 76±1% moisture content, a2- Flaccid root in about 70±1% moisture content, a3- completely flaccid root with 65±1% moisture content (Vukov and Hangyal 1985). Extraction method (B) included three levels: b1- cold digestion extraction method, b2- French method based on extract volume, and b3- two phase hot digestion method known as Parker method.

Tolerant cultivar to salt (MSC2×7233. P.29) in Rodasht, Isfahan and tolerant cultivar to drought (BP) were sown in the earliest proper time in spring with approximate density of 100000 plants per hectare in Kamalabad, Karaj. Roots harvested from each plot were spread on the ground. The first and fourth quartiles of the roots were selected and removed based on the root size and visual selection. The second and third bulk quartiles with medium size were chosen as an average plot criterion (Akeson 1980). A total of 180 samples collected from both Karaj and Isfahan regions were transferred to the Sugar Beet Technology Laboratory of Sugar Beet Seed Institute, Karaj. Each sample contained 30 to 40 single roots. Karaj samples consisted of 36 samples collected from non- stress condition and 36 samples from drought stress condition, and Isfahan samples consisted of 108 samples in which 36 samples were from non-stress condition, 36 samples from mild saline stress, and 36 samples from high saline stress conditions. The first 60 samples were washed and analyzed quickly, but for the second and third 60 samples, the roots were washed and the pulp was taken when the sample weight decreased to about 7 and 13% in open air, respectively. Of each root sample, at least 300 g completely uniform and mixed brei was prepared as a representative of the sugar beet root samples and different traits such as dry matter, pulp, Brix, extract electrical conductivity, and the brei density were measured, and using the equation 1, the extract volume of 26 g brei was determined (Van der Pol et al. 1998). Then, the remaining brei (300 g) was equally divided into three groups and each of them was placed in a special tray containing 4 parts, and each three trays received same number of treatment. 180 trays were used for extraction based on the conventional method (26 g of sugar beet brei with 177 ml lead super acetate). The second 180 trays were extracted based on the French method and the third 180 trays were extracted based on the hot digestion method and sugar content was measured for all three methods.

For all samples, sugar content was measured according to polarimety method, potassium and sodium were measured according to flame photometry method and amino N through water value using Betalyzer device (Kunz 2004). Dry matter was measured by placing a certain amount of brie in the oven at 105 ^{o}C until constant weight (Riyahi and Sajadi 1984). The pulp was measured through rising 25 g of sugar beet brie in three different phases and in each phase with 400 ml boiling water (Sheikholaslami 1997). Measurement of the soluble solids in the juice was done through placing the juice extracted from 20 g brie on refractometer device sensor at laboratory temperature (Sheikholaslami 1997). To measure the volume of the extract obtained from 26 g brei, French method and equation 1 were used (Van der pol et al. 1998). To measure density of the extract, 100 g brei that was mixed quite uniformly was extracted using press machine and was determined using picnometer at 25 ^{o}C through db=mj/vj relation (Sheikholaslami 1997).

In the above equation, mj is the brei juice weight based on g and vj is the volume change of picnometer. To apply a two-phase digestion method (Parker method), initially 26 g of sugar beet brei was weighed and mixed with 190 ml water at 80 ^{o}C for 30 min and then the solution was cooled and made up to 200 ml and sugar content was measured (P1). In the second phase, the remaining brei from the first phase was mixed with 180 ml water at 80 ^{o}C, clarified and made up to 200 ml and sugar content was measured (P2). Total sugar content was obtained from the following equation P=P1+P2 (Parker 1970). To measure the juice electrical conductivity, 6.5 ml of the juice brei was made up to 50 ml (Vukov 1977).

RESULTS AND DISCUSSION

Table 1 shows the analysis of variance for sugar beet roots produced with different origins. No significant difference was observed among extraction methods for the measurement of sucrose in the roots collected from non-stress conditions. Significant difference was found for the effect of extraction methods on the measurement of the sucrose, in the roots produced in semi-saline condition in Isfahan ($p<0.05$) and also for the roots

Table 1. Results of the analysis of variance on the effect of extraction method and root moisture content on sucrose content, measured for five groups of the sugar beet.

		Measured sucrose content sum of squares				
S.O.V	df	Sucrose content of the roots produced under non stress condition, Karaj	Sucrose content of the roots produced under non stress condition, Isfahan	Sucrose content of the roots produced under drought stress condition, Karaj	Sucrose content of the roots produced under semi-salt stress condition, Isahan	Sucrose content of the roots produced under high saline stress condition, Isahan
Extraction method	2	0.396^{ns}	0.225^{ns}	2.52**	3.63*	17.93**
Root moisture	2	144.7**	12.1**	187.2**	75.4**	29.63**
Root moisture×method	4	0.112^{ns}	0.387^{ns}	0.965^{ns}	0.035^{ns}	1.08^{ns}
Error	99	0.87	1.73	0.49	1.11	1.40
CV		4.74	6.99	4.04	6.00	6.25

ns=not significant, * significant at p<0.05, ** significant at P<0.001

Table 2. Grouping of the average sucrose content measured in different extraction methods in five groups of sugar beet.

Treatment	Parameters				
Extraction method	Sucrose content of the roots produced under non stress condition, Karaj	Sucrose content of the roots produced under non stress condition, Isfahan	Sucrose content of the roots produced under drought stress condition, Karaj	Sucrose content of the roots produced under semi-salt stress condition, Isahan	Sucrose content of the roots produced under high saline stress condition, Isahan
Conventional	19.79 a	18.93 a	17.69 a	17.95 a	19.23 a
Modified	19.69 a	18.81 a	17.30 b	17.45 b	18.03 b
Hot digestion	19.58 a	18.77 a	17.20 b	17.36 b	17.98 b

Means with the same letter in each column are not significantly different based on Duncan test at 0.05

Table 3. Grouping of the average root moisture treatments for some quality traits of sugar beet in non-stress condition in Karaj

Treatment	Parameters					
Root moisture content (%)	Sucrose (%)	Dry matter (%)	Brix (%)	Pulp (marc %)	Extract electrical conductivity (millisiemens/cm)	Volume of 26 g brei with 177 ml clear solution (ml)
72.5	17.50 c	24.50 c	19.10 c	4.8 c	0.85 c	200.0 a
72.8	20.15 b	27.2 b	22.80 b	5.3 b	0.90 b	199.4 b
70	21.43 a	30.0 a	24.90 a	5.9 a	0.98 a	199.1 c

Means with the same letter are not significantly different based on Duncan test at 0.05

produced under drought and saline stress conditions (p<0.01). The analysis of root moisture content for all the roots produced under non-stress and stress conditions (drought, saline, and semi-saline stress) in Karaj and Isfahan regions showed that the effect of root moisture content on sucrose content was significant (Table 1). Moisture content × extraction method interaction for sucrose measurement was not significant for the roots produced under non-stress, drought, saline and semi-saline stress conditions (Table 1).

Averages of the three extraction methods including cold digestion, modified method (French), and hot digestion showed that the sugar content percentage measured by conventional method (cold digestion) was significantly higher for the roots produced under drought stress condition in Karaj region and also for the roots produced under semi-saline and saline stress condition in Isfahan region compared with French modified method and hot digestion method. No significant difference was observed between modified extraction method and hot digestion method for these roots.

The effect of root moisture content for the roots produced in-non stress condition in Karaj showed that with moisture content reduction in the roots from 75.5 to 72.8 and 70%, the rate of sucrose, root dry matter, extract brix, pulp, and electrical conductivity of the extract increased significantly and the volume of the extract of 26 g brei with 177 ml clear solution decreased significantly (Table 3).

At the beginning of the experiment, the effect of root moisture content in non-stress condition in Isfahan region resulted in 72.7% lower moisture content in the roots than the roots produced at normal condition in Karaj owing to the climate conditions of the Project Site and the interval of the time since the roots were harvested and trans-

Table 4. Grouping of the root average moisture treatments for some qualitative traits in non-stress condition in Isfahan

Treatment	Parameters					
Root moisture content (%)	Sucrose (%)	Dry matter (%)	Brix (%)	Pulp (marc %)	Extract electrical conductivity (millisiemens/cm)	Volume of 26 g brei with 177 ml clear solution (ml)
72.7	19.50 a	27.3 a	22.8 b	4.7 c	1.3 c	199.6 a
72.5	18.50 b	27.4 b	22.5 b	5.4 b	2.2 b	199.3 b
72.0	18.51 b	28.0 b	23.5 a	5.7 a	2.4 a	199.3 b

Means with the same letter in each column are not significantly different based on Duncan test at 0.05

Table 5. Grouping of the root average moisture treatments for some qualitative traits in drought stress condition in Karaj

Treatment	Parameters					
Root moisture content (%)	Sucrose (%)	Dry matter (%)	Brix (%)	Pulp (marc %)	Extract electrical conductivity (millisiemens/cm)	Volume of 26 g brei with 177 ml clear solution (ml)
75.5	14.76 b	24.5 c	19.5 c	5.1 c	0.99 c	199.9 a
73.5	18.60 a	26.5 b	22.5 b	5.4 b	1.02 b	199.5 b
72.0	18.83 a	27.8 a	24.0 a	5.7 a	1.07 a	199.3 c

Means with the same letter in each column are not significantly different based on Duncan test at 0.05

Table 6. Grouping of the root average moisture treatments for some qualitative traits in semi-saline stress condition in Isfahan

Treatment	Parameters					
Root moisture content (%)	Sucrose (%)	Dry matter (%)	Brix (%)	Pulp (marc %)	Extract electrical conductivity (millisiemens/cm)	Volume of 26 g brei with 177 ml clear solution (ml)
73.0	15.93 b	27.0 c	22.7 b	5.6 b	2.2 c	199.4 a
71.6	18.18 a	28.4 b	22.5 b	6.1 a	2.8 b	199.2 b
70.0	18.63 a	30.0 a	26.2 a	6.2 a	3.0 a	198.9 c

Means with the same letter in each column are not significantly different based on Duncan test at 0.05

ferred to Karaj (Table 3 and 4). Storage of the toots in Isfahan when the average temperature was 5 ^{o}C caused a small decrease in moisture content from 72.7 to 72.5% and eventually to 72% (Table 4). However, storage of the roots in Karaj at the same condition resulted in moisture content reduction from 75.5% at the start of the experiment and finally decreased to 70% (Table 3).

The effect of root moisture content on some qualitative traits of the roots produced in drought stress condition in Karaj showed that with root moisture reduction from 75.5 to 73.5%, sucrose content increased significantly but this increase was not significant for the root moisture content reduction from 73.5 to 72%. Root dry matter, pulp, brix, and electrical conductivity of the extract parameters were increased significantly with root moisture content decrease from 75.5 to 73.5 and 72%, and the extract volume of 26 g brei with 177 ml clear solution decreased significantly (Table 5).

For the roots produced under semi-saline condition in Isfahan, the sucrose and pulp percentage increased significantly with root moisture reduction from 73 to 71.6% but no significant difference was found for the extract brix. No significant difference was found for sugar and pulp content with root moisture reduction from 71.6 to 70% but it was significant for the brix. The root dry matter and electrical conductivity of the extract increased significantly with root moisture reduction from 73 to 71.6 and 70% and the extract volume of 26 g brei with 177 ml clear solution increased significantly (Table 6).

Table 7 shows that with root moisture content reduction from 71.8 to 70%, sucrose percentage increased significantly but it was not significant for the pulp rate. The root moisture content reduction from 70 to 68.9% caused a significant increase in the pulp rate. However, it didn't cause a significant increase in sucrose rate. Dry matter, brix, and electrical conductivity traits were increased significantly with root moisture content reduction from 71.8 to 70 and 68.9%, and the extract volume of 26 g brei with 177 ml clear solution decreased significantly.

As Table 2 shows, there was not a significant difference between cold digestion method and French modified method or hot digestion method for the sucrose content in the roots produced under normal condition, however, differences were significant for the roots produced under semi-

Table 7. Grouping of the root average moisture treatments for some qualitative traits in saline stress condition in Isfahan

Treatment	Parameters					
Root moisture content (%)	Sucrose (%)	Dry matter (%)	Brix (%)	Pulp (marc %)	Extract electrical conductivity (millisiemens/cm)	Volume of 26 g brei with 177 ml clear solution (ml)
71.8	17.40 b	28.2 c	22.8 c	6.3 b	2.0 c	199.2 a
70.0	18.74 a	30.0 b	24.4 b	6.4 b	2.2 b	199.0 b
68.9	19.11 a	31.1 a	26.5 a	7.1 a	2.3 a	1989.6 c

Means with the same letter in each column are not significantly different based on Duncan test at 0.05

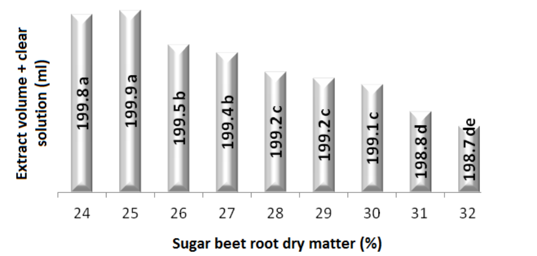

Fig. 1. The extract volume changes of 26 g brei with 177 ml clear solution owing to root dry matter variation.

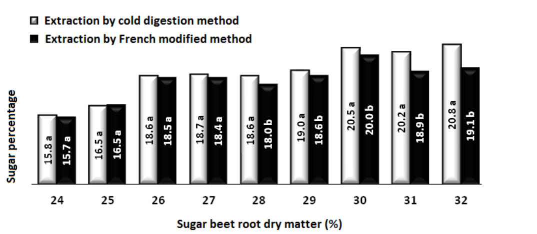

Fig. 2. Comparison of the sugar content measured by French modified method and cold digestion method

saline stress ($p<0.05$) and drought and saline stress ($p<0.01$) conditions. The observed difference could possibly be due to the increase of dry matter and pulp in the roots under stress condition. No significant difference was found between French modified extraction method and hot digestion method for any of the groups. According to Tables 4 and 7, the increase in sucrose, pulp, brix, and electrical conductivity was associated with an increase in root dry matter. When the dry matter increased from 24 to 32%, pulp (marc) also increased from 4.7 to 7.1%. Hirchumller and Krocher (1968) also reported same results.

The extract volume changes of 26 g brei with 177 ml clear solution showed that the roots with 24 and 25% dry matter had no significant difference but with dry matter increase from 25 to 26%, a significant difference was found.

Increase in root dry matter from 24 to 27% caused no significant difference between cold digestion method and modified method, while an increase from 27 to 28% made this difference significant.

Withdrawal of water from the roots after harvest until sugar extraction in factory is inevitable. With root weight reduction and beginning of the flaccidity, the root sugar content increases. This is normal since concentration is a function of the solution. In this study, the effect of flaccidity on the extract volume difference from 200 ml was investigated and the results showed that there was no significant difference between the hot digestion and French modified methods, and the French modified method is recommended compared with hot digestion method due to the ease of implementation. However, both of these methods showed significant difference with conventional cold digestion method for the roots produced under stress. Cold digestion and French methods showed no significant difference for the roots with dry matter between 24 to 27%. According to the results of this study and the simplicity of the cold digestion method, it is recommended to use cold digestion method for the roots with dry matter up to 27% and modified French method for the roots with dry matter more than 27%.

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