

## Accuracy of Sugar Analyses of Honey by the Selective Adsorption Method

The selective adsorption method for determining sugars of honey (1, 2) has recently been adopted first action by the Association of Official Agricultural Chemists (3). In this procedure honey sugars are divided by charcoal column adsorption before analysis into three groups: monosaccharides, disaccharides, and higher sugars. Each fraction is analyzed for individual sugars by modifications of conventional volumetric methods.

In the development of the method, known sugar mixtures were subjected to the procedure and recoveries calculated. Additions of known sugars to honey solutions were satisfactorily accounted for.

The selective adsorption procedure has been used in the analysis of over 500 samples of honey from all parts of the United States. During this work opportunities were taken to obtain measures of the accuracy of the method. Aliquots of the three analytical fractions for each of 17 consecutive samples were evaporated and the dry weight was compared with that calculated from the sugar analyses. The results demonstrate the general accuracy of the method and also give some information about the materials not analyzed by the procedure.

The accuracy of the method as applied to honey monosaccharide fractions from the routine analyses of five honey samples was also checked by analyzing for dextrose and

levulose polarimetrically as well as by the chemical procedure. While it has been shown (4) that polarimetric determination of fructose in honey is not accurate, charcoal column pretreatment removes interfering sugars and other materials and provides a solution containing only dextrose and levulose which can be analyzed polarimetrically.

### Methods and Results

In the analytical procedure, the carbohydrates of a honey sample (0.8–1.0 g) are separated as follows:

Fraction A—250 ml—dextrose, levulose

Fraction B—250 ml—sucrose, reducing disaccharides

Fraction C—100 ml—higher sugars

The dextrose and levulose are determined individually. Reducing disaccharides are determined in Fraction B without preliminary hydrolysis and calculated as maltose; sucrose is determined by increase in reducing power after a mild acid hydrolysis. In Fraction C, reducing sugars after hydrolysis are determined by copper reduction and reported as dextrose.

Fifty ml aliquots of each of these three fractions from 17 consecutive honey samples were evaporated to dryness in a current of air on a steam bath and the weights of the residues determined. All solutions and residues were colorless. Table 1 shows the weights obtained for four representative

\* Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

Table 1. Weight of material in 50 ml aliquots of analytical fractions

Fraction	Sample A		Sample B		Sample C		Sample D	
	Found (Mg)	Calc.						
Monosaccharide	140.3	138.9	136.8	137.6	135.0	134.7	132.1	132.2
Disaccharide	18.9	14.2	23.4	19.6	20.8	17.3	17.6	15.9
Higher sugars	7.0	5.6	10.0	8.2	9.7	6.9	10.5	8.0

**Table 2. Analysis of variance for data in Table 1**

Source of Variance	DF	Monosaccharides			Disaccharides			Higher Sugars		
		SS	MS	F	SS	MS	F	SS	MS	F
Materials	16	896.8	56.0	2.86	163.1	10.2	11.2**	328.1	20.5	25.7**
Methods	1	2.18	2.18	0.11	58.8	58.8	64.9**	54.0	54.1	67.7**
Error	16	313.3	19.6		14.5	0.91		12.8	0.80	
Total	33	1212.28			236.4			349.9		

\*\* Significant at .01 probability level.

**Table 3. Percentages of material in analytical fractions, determined by two methods, whole sample basis**

Fraction	Sample A		Sample B		Sample C		Sample D		Average 17 Samples	
	By Wt.	By Anal.	By Wt.	By Anal.						
Monosaccharide	71.37	70.67	69.68	70.12	67.82	67.70	69.97	70.03	71.23	71.06
Disaccharide	9.61	7.22	11.92	9.99	10.45	8.60	9.32	8.40	9.12	7.73
Higher sugars	1.62	1.15	2.16	1.68	1.95	1.38	2.22	1.70	2.18	1.22
Total sugars	82.60	79.04	83.76	81.79	80.22	77.68	81.51	80.13	82.53	80.01
Moisture*	15.7	15.7	15.8	15.8	18.2	18.2	18.0	18.0	17.3	17.3
Total	98.3	94.7	99.6	97.6	98.4	95.9	99.5	98.1	99.8	97.3
Not analyzed	1.7	5.3	0.4	2.4	1.6	4.1	0.5	1.9	0.2	2.7

\* Moisture content of honey sample.

samples, together with the weight calculated from the chemical analyses. An analysis of variance on the individual weights of the three fractions from the 17 samples, found by weighing and calculated from the analytical values, is shown in Table 2. The difference in the results for Fraction A by the two methods is not significant, whereas the amount of unanalyzed material in both Fractions B and C is highly significant.

Table 3 shows the amount of material found in the fractions by evaporation and the amount calculated from the analyses. Both amounts were calculated for the entire sample. The last line [100 - (total material + water)] is the material not accounted for by each procedure. About 2.5% of honey material (17-sample average) in the three analytical fractions escapes analysis by the selective adsorption procedure. The distribution of this material among the three fractions is given in Table 4. It can be seen

that the largest part of the material is in Fraction B—the disaccharides.

For the polarimetric determination of the sugars of Fraction A, 100 ml aliquots of Fraction A from five successive honey analyses were evaporated as before. They were made to 10.00 ml with water and a little

**Table 4. Distribution of unanalyzed material on whole sample basis\***

Fraction	Sample				17-Sample Average
	A	B	C	D	
Mono-saccharide	0.70	-0.44	0.12	-0.06	0.40
Di-saccharide	2.39	1.93	1.85	0.98	1.40
Higher sugars	0.47	0.48	0.57	0.52	0.52
Total	3.56	1.97	2.54	1.44	2.32

\* Values show amount of unanalyzed material in each fraction, as per cent of entire sample.

ammonia, and their rotation was determined. The specific rotation was calculated using the evaporated weights, and the composition of the solution was calculated from the known values for pure levulose and dextrose. An example is as follows:

Sample E (Table 5). Original weight = 0.9958 g

Residue from 100 ml Fraction A = 0.2806g  
Angular rotation (2 dm) =  $-1.55^\circ$

$[\alpha]_D^{20} = -27.62^\circ$

$[\alpha]_D^{20}$  levulose =  $-92.5^\circ$ ; dextrose =  $52.5^\circ$

$$\frac{-92.5 - (-27.62)}{-92.5 - (52.5)} = \frac{-64.88}{-145.0} = 44.74\% \text{ dextrose}$$

$$0.2806 \times .4474 = .1255 \text{ g dextrose}$$

$$.2806 \times (1 - .4474) = .1550 \text{ g levulose}$$

$$\frac{.1255 \times 2.5 \times 100}{.9958} = 31.51\% \text{ dextrose}$$

$$\frac{.1550 \times 2.5 \times 100}{.9958} = 38.91\% \text{ levulose}$$

Found by selective adsorption method:

31.19% dextrose,

39.15% levulose.

Table 5 shows the values so obtained for the five samples, and Table 6 shows an analysis of variance of these data. It can be seen that the variance is almost entirely due to materials (different honey samples) and that due to the methods is not significant at the 5% level for either dextrose or levulose. ( $F = 6.4$  and  $0.33$ ; critical values at the 5% level =  $6.39$  for materials and  $7.71$  for methods).

#### Discussion

The agreement between the values ob-

**Table 5. Determination of dextrose and levulose in monosaccharide fractions by two methods**

Sample	Dextrose		Levulose	
	Chemical	Polarimetric	Chemical	Polarimetric
E	30.79	31.51	39.15	38.91
F	33.57	34.57	37.55	36.55
G	33.15	33.87	38.82	38.40
H	29.47	30.22	38.69	39.77
I	33.52	33.21	38.65	38.24
Av.	32.10	32.68	38.57	38.38

tained by weighing and by calculation from the dextrose and levulose values in the monosaccharide fraction is satisfactory. This fraction is the most important in honey and makes up about 85% of the sugars. The 0.40% discrepancy found for the 17-sample average (Table 4) can be compared with the standard deviation obtained when four honey samples were analyzed by three analysts in one laboratory (0.38% for dextrose, 0.42% for levulose) (3).

The method of analysis for Fraction B is a compromise, since it has been found to contain maltose, isomaltose, turanose, maltulose, sucrose (5), and also kojibiose (6). There is also some evidence of trehalose (5) and leucrose (6). The relative reducing power of these sugars varies considerably; kojibiose is reported to have only about 6% of the reducing power of glucose toward the Shaffer-Hartman copper reagent (7). Trehalose, being non-reducing, would not be determined by the procedure used, but would appear in Fraction B if present. It is therefore likely that the un-analyzed material in the disaccharide fraction is at least in part

**Table 6. Analysis of variance of data in Table 5**

Source of Variance	DF	Dextrose			Levulose		
		SS	MS	F	SS	MS	F
Materials	4	26.08	6.52	48.5**	5.87	1.47	4.90
Methods	1	0.83	0.83	6.4	0.10	0.10	0.33
Error	4	0.52	0.13		1.19	0.30	
Total	9	27.43			7.15		

\*\* Significant at .01 probability level.  $F_{.05} = 6.39$  for materials;  $7.71$  for methods.

kajibiose; it may be seen from Table 3 to vary from sample to sample.

The un-analyzed material in Fraction C averages 0.52%. Inspection of the 17 samples shows that it does not vary as widely as does that in Fraction B. It may be a systematic error in the determination which is due to incomplete hydrolysis of higher sugars or destruction of fructose in the acid hydrolysis.

The essential accuracy of the analytical procedure is evidenced by the satisfactory agreement for dextrose and levulose values in the monosaccharide fraction by the two methods, plus the agreement between weighed and calculated residues. An earlier study of five methods of honey analysis made prior to development of the selective adsorption method (4) showed that variance due to methods was highly significant and greater than that due to differences among honey samples of different floral types. Here, Table 5 shows that variance due to samples is about ten times that due to methods in the analysis of monosaccharide fractions by two procedures (chemical and physical). Variance due to methods is not significant at the 5% level for either dextrose or levulose.

#### Summary

1. Comparison of dry weights of fractions

from the selective adsorption analysis of honey with values calculated from the analysis shows that about 2.5% of the material passing through the charcoal column is not analyzed.

2. Most of this material is in the disaccharide fraction and probably represents kojibiose, and possibly also trehalose.

3. Polarimetric analyses of the monosaccharide fraction from the honey analyses gives results for dextrose and levulose not differing significantly from those obtained by chemical methods.

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