

FOOD ADULTERATION

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Detection of Beet Sugar Adulteration of Honey

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Quantitation of oligosaccharide-bound galactose by galactose oxidase treatment of the higher sugar fraction is useful to screen honeys with normal stable carbon isotope ratio values for the presence of beet sugar products. For 23 beet sugar products tested, the mean bound galactose value was 30.1 mg/100 g (as galactose); for 81 honeys, the mean was 3.1 mg/100 g, $s = 4.4$. Nine percent of the honey samples tested had values in the beet sugar range, so additional testing by other procedures is required for confirmation of adulteration, i.e., samples with 8–80 mg/100 g bound galactose should be further tested.

The undeclared addition of other sugar products to honey has been a concern of industry for at least 100 years. As improved procedures for detecting addition have become available, advances in sweetener technology have produced more highly refined, less detectable syrups. A major threat to the integrity of honey in the market (and also to other food products) was the development of high fructose corn syrup (HFCS), undetectable by any then known test when mixed with honey. The development of stable carbon isotope ratio testing made it possible unequivocally to demonstrate moderate levels of corn syrups in honey (1) and maple syrup (2) as well as undeclared addition of HFCS to citrus, apple, and other juices (3). The test also detects cane sugar addition because cane and corn plants both use the Hatch-Slack photosynthesis cycle. Unfortunately, the test is useless for differentiating beet sugar. The beet is a Calvin-type plant, the same as honey-producing plants and most fruits and vegetables; hence, the isotope ratio values do not differ sufficiently for detection purposes.

Beet sugar addition can be detected, but the procedure requires rather extensive analytical work and is too expensive to apply routinely. A screening test to select candidates for further examination or, ideally, to stand alone, is needed to enhance the defense against honey adulteration.

We developed a procedure using galactose oxidase which has considerable potential to indicate the presence of beet sugars and syrups in honey. Commercially available galactose oxidase from *Dactylium dendroides* will oxidize galactose and galactose derivative with a free 6-hydroxyl group. It therefore can be used to measure galactose, melibiose, raffinose, stachyose, planteose, and other oligo- or polysaccharides with a terminal galactose (4).

This enzyme oxidizes the 6-hydroxyl of galactose and related compounds (D-talose, 2-deoxy-D-galactose, D-galactosamine, N-acetyl-D-galactosamine) to produce an aldehyde group and hydrogen peroxide. Avigad et al. (4) used dianisidine as the chromogen in the reaction; Fischer and Zapf (5) demonstrated that this compound competitively inhibits the galactose oxidase and recommended *o*-cresol as a suitable chromogen. Although raffinose is the oligosaccharide of interest in beet sugar, the capability to include other oligosaccharides may be useful.

To evaluate the reaction for differentiating beet sugar and honey, we applied it to the "higher sugar" fraction from 23 beet sugar products and 85 honeys. Removal of mono- and disaccharides and concentration of the oligosaccharides (higher sugars) by charcoal column treatment increases the sensitivity of the procedure.

METHOD

Reagents

(a) *Galactose oxidase*.—Type V (Sigma Chemical Co., No. G3385), 300 units/mL water. Reagent is described by Fischer and Zapf (5).

(b) *Phosphate buffer*.—0.1M pH 7.0, containing 55 mg *o*-cresol and 40 mg peroxidase (Sigma, Type 1, P-8125)/100 mL water.

Procedure

Prepare charcoal column using Darco G-60 as directed in 31.148. Weigh, to nearest mg, 1 g sample in 30 or 50 mL beaker. Add 10 mL water to dissolve and place solution on top of column. Force into column with suction but do not let column run dry. Rinse beaker with two 5 mL portions of water and force rinses into column. Wash column with 300 mL 7% ethanol, which is discarded, and then with 100 mL 50% ethanol. Evaporate eluate on steam bath in current of air to dryness, and add 5.00 mL water by pipet. Transfer 0.75 mL portions to 2 test tubes and 0.75 mL portions of a solution containing 1.30 mg galactose/100 mL to 2 test tubes. Place tubes in rack in the order: standard, sample, sample, standard. Add 0.10 mL buffer and 0.05 mL galactose oxidase solution to each (vortex mixer), and let stand 30 min at room temper-

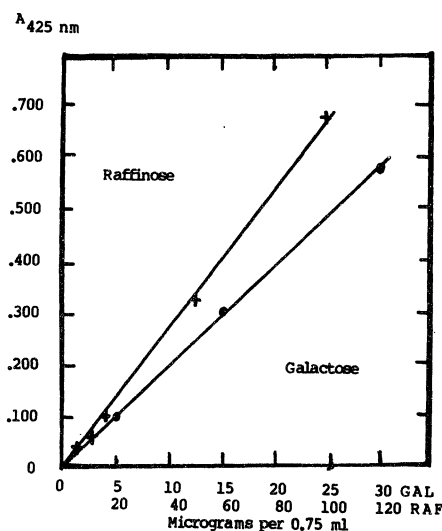


Figure 1. Determination of galactose and raffinose with galactose oxidase.

Table 1. Reproducibility of galactose oxidase treatment applied to beet sucrose

Run	Absorbance, 425 nm	Equiv. galactose, μg	Bound galactose, mg/100 g
1	0.491	22.7	30.0
2	0.489	22.6	30.1
3	0.485	22.5	30.0
4	0.491	22.7	30.3
5	0.481	22.3	29.7
6	0.479	22.2	29.6
Mean		22.5	30.0
SD		0.21	0.30
CV, %			1.0

ature. Immediately determine absorbance at 425 nm. Average readings of samples (A) and of standard (A').

Calculation

Calculate as follows:

$$\text{Bound galactose (BG) in tube, } \mu\text{g} = (\mu\text{g galactose in std tube}) \times A/A'$$

$$\text{BG, mg/100 g sample} = \mu\text{g BG in tube} \times 0.667$$

where factor 0.667 = 5 (eluate vol.)/0.75 (sample vol.) \times 100 g/1000 μg

Experimental

Time of incubation.—A solution of 15 μg galactose in 0.75 mL water was incubated at room temperature with 0.10 mL buffer and 0.05 mL galactose oxidase solution and absorbance was measured at 5-min intervals. The maximum value of 0.300 was obtained from 25 to 40 min after mixing.

Calibration.—Using 30-min incubation, several calibrations were carried out using galactose and raffinose. As shown for each in Figure 1, response was linear over the range 0–30 μg galactose/0.75 mL and 0–120 μg raffinose/0.75 mL. Calibrations at different times produced slightly differing results, so a standard solution should be included with each run, rather than using a factor, to eliminate effects of variable room temperature.

Reproducibility.—Six 0.75 mL aliquots of galactose solution containing 16 μg /0.75 mL were analyzed as above. Absorbance values were 0.339, 0.332, 0.337, 0.344, 0.330, and 0.335, for a mean of 0.336, $s = 0.005$, equivalent to 0.25 μg galactose.

Pretreatment of sample.—Two existing procedures (6) for concentrating oligosaccharide fraction were compared: method 31.123, using 17 cm column of charcoal with successive washes by 1, 7, and 50% ethanol solutions, and method 31.148 (detection of corn syrup in honey by thin layer chromatography (TLC)), using 8 cm bed with washes of 7 and 50% ethanol. The latter procedure saves considerable time, because the fraction of interest is eluted by 50% alcohol. One gram sam-

ples each of honey and beet sucrose were treated by both procedures; the use of the shorter column was quite satisfactory.

Reproducibility of procedure.—Six 1 g samples of beet sugar were passed through the above procedure and the evaporated eluate was diluted to 10 mL. Results of the analysis of 0.75 mL portions of each are shown in Table 1. A coefficient of variation of 1% was calculated.

Recovery of bound galactose in beet sugar added to honey.—Increments of beet sucrose containing, by analysis, 29.9 mg/100 g of bound galactose were added to 1 g samples of honey. Results for the analysis of each sample are shown in Table 2. Recovery ranged between 92.4 and 102%, quite adequate for the purpose of screening honey for beet sugar.

Bound galactose content of beet sugar products and of honey.—Various beet sugar products were obtained from 4 companies, representing 11 factories in 6 states. Thirteen samples of refined granulated sucrose, 5 of liquid sucrose (67 Bx), and 5 of 50% invert syrups (77% solids) were provided. Each was analyzed by the procedure described above, after the final solution was diluted as needed to keep absorbance values below 0.6. Table 3 shows the results. The average value for all 23 samples is 30.1 mg/100 g dry basis, $s = 17.3$.

Eighty-five samples of honey from several collections were analyzed for bound galactose. These were 8 retail samples, 31 unprocessed samples produced in 1982 by members of the Sioux Honey Association, and 46 samples from a USDA collection which had been stored at 0°F since their analysis for stable carbon isotope ratio in an earlier study (1). These had been selected originally to represent all commercially significant types and areas of the United States, from the 1974–1975 crops. Their isotope ratio values are on record (7). Distribution of the values for honey is shown in Figure 2. Several of the samples had bound galactose contents far in excess of all others and also of the beet sugars. The individual values for all honeys and their floral types and location of production appear elsewhere (8). The mean for 81 of the honey samples was 3.1 mg/100 g, $s = 4.4$. Four very high values were excluded from the calculation (8).

Bound galactose content of fruit juice concentrates.—Beet sugar when added to fruit juice concentrates is also undetectable by isotope ratio. During the work described here, 3 commercial fruit juice concentrates were analyzed by the procedure described. Results were as follows: 3.0 mg/100 g in orange juice concentrate brand A; 1.7 mg/100 g in orange juice concentrate brand B; 10.3 mg/100 g in apple juice concentrate. Further investigation of this material in orange juice concentrate may be worthwhile.

Discussion

The objective of this work was to develop a screening test to select honey samples which may contain added beet sugar. Ideally, such a test would be self-conclusive, but even the

Table 2. Recovery of bound galactose from beet sugar in mixture with honey

Beet sucrose in sample, g ^a	Absorbance, 425 nm	Bound galactose, μg		Diff., μg ^b	Rec., %
		Found	Added		
0	0.096	34	0	0	—
0.200	0.265	94	59.8	60	100.3
0.400	0.440	156	119.6	122	102.0
0.600	0.593	210	174.9	176	98.1
0.800	0.720	255	239.2	221	92.4

^aIndicated amount of beet sucrose (containing 29.9 mg bound galactose/100 g) added to 1.000 g honey.

^bBound galactose found, minus that from honey (34 μg).

Table 3. Bound galactose content (mg/100 g) of refined beet sugar products

Company	Factory	Granulated sucrose	Liquid sugar ^a (67 Bx)	50% Invert ^a (77% solids)
A	1	21.3	18.9	11.0
B	1	30.3	39.8	12.6
C	1	74.7		
C	2	45.4		
C	3	48.7		
C	4	26.7		
C	5	17.3	21.9	19.1
D	1	44.7		
D	2	42.7	37.2	23.4
C	6	11.7		
C	7	8.8	15.2	16.5
B	2 ^b	56.0		
B	2 ^c	48.7		
Mean		36.7	26.6	16.5
SD		19.2	11.2	5.0

All data: Mean, 30.1; s, 17.3

^aData calculated to dry basis.

^bBeginning of campaign.

^cEnd of campaign.

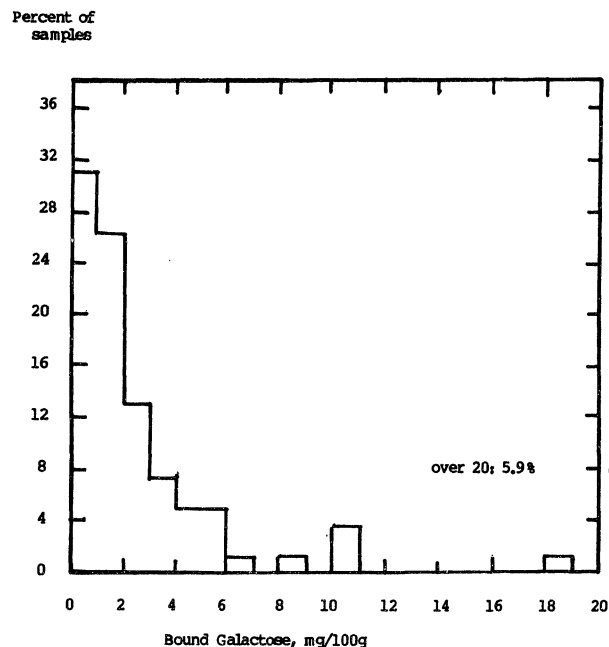


Figure 2. Distribution of bound galactose values for 85 honey samples.

stable carbon isotope ratio determination is inconclusive for the presence of cane or corn sugars under certain circumstances. A thin layer chromatographic test for corn syrup is required for discrimination of pure honeys with $\delta^{13}\text{C}$ values between -23.4 and -21.5% and those containing low levels of HFCS (9). The presence of 3 honeys with very high values (215, 98.6, 786), far higher than any beet sugar product, and the occurrence of 2 more values that are near the mean for beet sugars prevent this procedure from being a single conclusive test. The value of 786 mg/100 g was obtained for a sample of tulip poplar honey (*Liriodendron tulipifera*). To learn if this is characteristic of this honey type, 2 additional samples were obtained from different sources; these showed bound galactose contents of 179 and 85; they do not appear in Figure 2. All 3 values far exceed those of any beet sugar products analyzed, as do 2 other values found for honey. The possible origin of the bound galactose in honey is discussed elsewhere (9).

It appears reasonable that samples with more than 8 and less than 80 mg/100 g galactose are possibly adulterated and

should be tested further by other analyses (10). Eight of 85 honeys tested (9%) were in this group. Such samples should be analyzed for fructose, glucose, sucrose, HMF (11, 12), and possibly proline and titratable acidity.

The preliminary cleanup and concentration is identical with that required for TLC analysis of high fructose and conventional corn syrups (13), so these tests could be carried out on the same eluate from the column. The TLC test is more sensitive than the isotope ratio test because of the spread of values in the latter for pure honeys, but the former does not detect cane or beet syrups. To detect all 3 sugar types in honey, isotope ratio and bound galactose plus confirmatory data are needed.

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REFERENCES

- White, J. W., Jr., & Doner, L. W. (1978) *J. Assoc. Off. Anal. Chem.* **61**, 746-750
- Morselli, M.-F., & Baggett, K. L. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 22-24
- Krueger, H. W., & Reesman, R. H. (1982) *Mass Spec. Rev.* **1**, 205-236
- Avigad, G., Ameral, D., Asensio, C., & Horecker, B. L. (1962) *J. Biol. Chem.* **237**, 2736-2743
- Fischer, W., & Zapf, J. (1964) *Z. Physiol. Chem.* **337**, 186-195
- Official Methods of Analysis* (1984) 14th Ed., Arlington, VA, secs 31.130, 31.135
- White, J. W., Jr., & Doner, L. W. (1978) *J. Apic. Res.* **17**, 94-99
- White, J. W., Meloy, R., Probst, J., & Huser, W. (1985) *J. Apic. Res.*, in press
- White, J. W., Jr (1980) *J. Assoc. Off. Anal. Chem.* **63**, 11-17
- White, J. W., Jr (1980) *J. Assoc. Off. Anal. Chem.* **63**, 1168
- White, J. W., Jr (1979) *J. Assoc. Off. Anal. Chem.* **62**, 509-514
- White, J. W., Jr., & Siciliano, J. (1980) *J. Assoc. Off. Anal. Chem.* **63**, 7-10
- Kushnir, I. (1979) *J. Assoc. Off. Anal. Chem.* **62**, 917-920