DOES RAFFINOSE OCCUR IN HONEY?

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SUMMARY

We report results of the analysis of 87 honey samples by a procedure we developed to indicate the presence in honey of syrups derived from beet sugar. Measurement of terminal galactose residues in the oligosaccharide fraction ("bound galactose") by a specific enzymic procedure shows all samples examined to contain this entity; the average value for all honeys analyzed was 3.1 mg (as galactose) per 100 g honey. Beet sugar products averaged tenfold higher. Raffinose is the only commonly-occurring sugar responding to the conditions of the analysis. Whether the raffinose is a nectar constituent or arises from traces of honeydew is not clear, though the latter appears more likely.

INTRODUCTION

Reports have appeared from time to time describing the identification of the trisaccharide raffinose in honey. Goldschmidt and Burkert (1955) so reported, as did Pourtallier (1968). Siddiqui (1970) reviewed his extensive research on honey sugars and and concluded that evidence for the presence of this sugar was not convincing; they depended largely upon chromatographic data alone. He suggested that previous workers may have confused it with a rare trisaccharide, theanderose (which contains no galactose), which he found in honey at 2.7%. Hadorn, Zurcher, and Strack (1974) also reviewed the question and were unable to demonstrate it unequivocally. Battaglini and Bossi (1972) agree that evidence for raffinose in honey is lacking.

The occurrence of raffinose in honeydews appears to be better established; Hadorn et al.(1974) found up to 2% raffinose in "Waldhonig", and were unable to demonstrate its

presence in honey. Lombard et al.(1984) using gas chromatography and enzymic hydrolysis reported small unspecified amounts of raffinose in honeydew of six aphid species. Maurizio (1975), reviewing the carbohydrates of honeydew, noted that "occasionally" raffinose is found therein.

In an effort to find ways to demonstrate the addition of beet sugar products to honey, we have analyzed the "higher sugar" (trisaccharide and higher) fractions of 23 beet sugar products and 88 honeys using a galactose oxidase reagent that oxidizes terminal galactose molecules having a free 6-hydroxyl group (Avigad et al. 1962). The reagent is specific for free galactose, raffinose, stachyose, planteose, and other oligosaccharides with a terminal galactose. Several related compounds are also oxidized (d-talose,2-deoxy-d-galactose, d-galactosamine, N-acetyl-d-galactosamine), but none of these would be found in the higher sugar fraction.

It was hoped that sufficient difference between beet sugar products and honey in bound galactose content would be found so that this single test would suffice for demonstrating adulteration of honey by beet sugar. Although the mean value for beet sugars was ten times that of the honeys, the extent of scatter of values for honey makes it necessary to confirm it by other means.

MATERIALS AND METHODS

Two groups of honey samples were used: samples representing honey produced in 1982 by members of the Sioux Honey Association, and a selection of 46 samples of U.S. honey from the 1974 and 1975 crops that had been collected by the U. S. Department of Agriculture for a study of the detection of honey adulteration. These had been stored at $0^{o}F$ for the entire period between collection and the analyses reported here. They were part of the 84 samples selected from nearly 500 to represent all commercially significant U. S. honeys and production areas. The isotope ratio values of

these samples are in the literature (White and Doner, 1978). In addition, a few samples of retail processed honey were included.

Full detail on the development and testing of the galactose oxidase procedure for honey analysis appear elsewhere (White et al. 1985). A one gram sample of honey is placed on a charcoal column, washed with 7% alcohol to remove mono- and disaccharide sugars, and eluted with 50% alcohol. The eluate is concentrated and analyzed with the galactose oxidase reagent of Fisher and Zapf (1964). The procedure may be standardized with galactose or raffinose. Since it is not known whether raffinose alone is being measured, results are reported as mg bound galactose per 100 g honey.

RESULTS AND DISCUSSION

Results of the analyses are shown in the table. The mean for all honeys is 3.1 mg/100g, but several fell between 10 and 20. Three very high values far exceeded any found for beet sugar and were excluded from the calculations. In order to learn if the result for the tulip poplar (Liriodendron tulipifera) honey was an aberration, two additional samples of this type were analyzed, giving 179 and 85 mg/100g. It is likely that these elevated bound galactose values are characteristic of this honey which has a composition somewhat differing from the average honey (White et al.1962). Calculating the 786 mg/100 g found for this honey to the equivalent amount of raffinose (according to our calibration, White et al.1985) gives 2360 mg/100 g, or 2.36% raffinose; the other two smaples of this type analyzed 0.53 and 0.25% as raffinose.

All samples tested showed some amount of bound galactose; values of 0.2-0.3mg/100g (2-3 ppm)found for a few of the honeys probably represent the minimum detectable by the procedure. Whether bound galactose (in raffinose) is a constituent of all honeys or simply represents small and variable amounts of contained honeydew is not clear. No

correlation was found between the bound galactose levels of those samples with higher amounts of bound galactose and the optical polarization of the samples; polarization values more positive than -2 degrees S are considered to be honeydews (White, 1980). The mean value of polarization for the 454 levorotatory samples from which the 46 USDA samples were taken was -14.7^{o}S. (White,1980). For the three samples with the highest value for bound galactose, the rotations were -2.5, -3.0, and -5.9^{o}, indicating a considerable honeydew content.

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Table 1. Bound galactose content (mg/100 g) of honey.

Location (State)	Floral Type	Bound Galactose	Location (State)	Floral Type	Bound Galactose
		SIOUX SAMPLES			
NE	Clover blend	0.3	ND	Clover-sunflower	1.0
ND	Clover blend	6.0	ND	Clover blend	3.3
ND	Clover-sunflower	0.7	ND	Sunflower blend	2.8
ND	Clover-sunflower	0.8	ND	Clover-sunflower	0.8
MT	Clover	0.2	SD	Clover blend	0.5
MT	Clover	0.5	ND	Clover	0.8
MN	Clover	2.2	MN	Blend	3.5
SD	Clover	1.3	NE	Clover	0.3
ND	Clover-sunflower	0.3	MN	Clover blend	0.3
SD	Clover blend	1.2	SD	Clover	1.3
MN	Clover-sunflower	1.7	MN	Clover-sunflower	1.7
MN	Clover-sunflower	1.7	MN	Clover-sunflower	2.7
MN	Clover-sunflower	1.2	MN	Clover-sunflower	2.2
SD	Clover	0.8	MN	Clover blend	1.7
ND	Blend	46.7[a]	MN	Blend	5.0
MN	Sunflower blend	3.3			
		USDA SAMPLES			
FL	Pepper tree	5.4	SD	Alfalfa	0.8
WI	Basswood	1.5	NE	Clover	10.5
ID	Alfalfa-sunflower	1.7	FL	Citrus	4.5
FL	Tupelo	4.4	FL	Citrus	5.3
FL	Palmetto	3.5	AR	Clover	2.5
TN	Locust	18.3	GA	Titi	2.3
MI	Clover	1.7	OH	Clover blend	0.9
TX	Huajillo	1.0	MT	Clover-alfalfa	1.2
TX	Tallow tree	1.5	SD	Clover	1.2

ND	Sweetclover	1.6	WI	Clover	1.3
WA	Spearmint	0.7	CA	Wild buckwheat	3.9
TX	Mesquite	10.3	CA	Safflower	2.6
IA	Trefoil	1.6	AZ	Alfalfa	1.5
MN	Soybean	2.9	WA	Blackberry-Thistle	7.0
AZ	Catclaw	1.4	IL	Clover	1.2
IN	Clover	14.5	NY	Clover	2.7
NC	Tulip poplar	786[a]	CA	Lima bean	1.3
OR	Vetch	5.7	OH	Aster	8.7
NE	Alfalfa	0.5	OK	Cotton	10.7
ND	Sunflower	0.7	MI	Blend	0.4
IA	Alfalfa Blend	1.7	MN	Blend	215[a]
UT	Alfalfa Blend	98.6[a]	OR	Thistle-Vetch	28.2
WY	Alfalfa-sweetclover	1.2	LA	Blend	3.1

RETAIL SAMPLES

Clover	1.0	Clover	0.8
Clover	1.0	Orange	2.5
"Natural"	5.0	"Natural"	1.5
Orange	2.8	"Generic"	0.7

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[a]Excluded from calculations.

Mean 3.1 mg/100g. Standard deviation 4.4.