

Preliminary Analysis of NC and Store Honey Samples for Pollen Content and Purity

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Introduction

Honeybees are a vital part of the U.S. agricultural ecosystem because roughly one-third of food consumed by humans is bee pollinated. The approximately 157 million pounds of honey produced by bees in 2015 and used for human food is a hardly trivial "byproduct" of their pollination activity. This occurred despite colony collapse disorder and various other issues affecting honeybee hives throughout the country.

Consumers are concerned with the quality and purity of honey that is available for sale in the U.S. The USDA has basic regulations about honey (USDA, 1985) but these are not particularly stringent compared to other countries. We used several simple ways to evaluate the quality of honey plus extracted pollen from honey to understand the source vegetation from which the bees obtained the pollen and nectar.

As part of a larger project in informal science education for middle and high school students about bees and gardens at UNC-Pembroke, we have begun evaluation of honeys. All the analysis of basic physical properties and the majority of the pollen analysis has been done by 14-17 year old students in our project. In our initial phase, we have examined three honeys:

A) Big Box Retailer B) Bolton, Columbus County C) Knightdale, Wake County



Figure 1: Approximate geographic locations for Big Box Retailer (A), Columbus County (B), and Wake County (C; suburban Raleigh).

Tests for Basic Physical Characteristics and Purity

Three simple tests (My Beeline, n.d.) allow us to determine basic information about the viscosity (resistance to flow) of the honey (Thumb test), general water content (Flame test), and specific gravity and solubility (Water test). These all indicate honey purity.

We also used a panel to evaluate the taste of the three honey samples. Honeys were identified by letter to avoid bias.

Thumb test

Place a drop of honey on your thumb nail with your finger level. If the honey runs off your finger and appears watery, then the honey is not viscous and probably not pure. If the honey stays in place and does not move, then the honey is pure.

Flame test

Dip cotton swab into a sample of honey. Once there is a large amount of honey on the cotton tip, carefully light the honey with a match. If the honey extinguishes immediately, that means that there is considerable water in the honey and that it is not pure (25% or more water). If the honey continues to burn, then the honey has relatively little water.

Water test

Drip the honey into a cup of water. If the honey disperses into the water and does not clump or stay uniform, then the honey is not pure. Watch for bubbles and honey floating back up to the top of the water. These characteristics can also note pure honey.

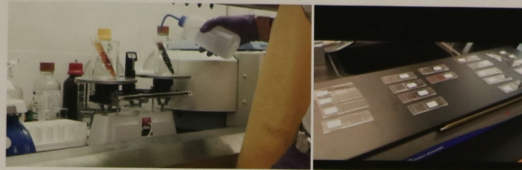


Figure 2: Preparing the honey samples for slides for pollen counts. Left hand photo shows samples after acetolysis just before washing commences.

Pollen Extraction

Pollen extraction used the procedure of Jones & Bryant (2014). Briefly, 10-20 g of honey is diluted with 100 ml 95% ETOH and two *Lycopodium* tablets are added. The resulting mixture is centrifuged in 15 ml tubes and the supernatant decanted until all the honey-ETOH mixture is removed and the residue is concentrated at the bottom of one tube.

This organic residue is then acetolyzed (90% acetic anhydride; 10% concentrated sulfuric acid) for 12 minutes in a hot water bath. After centrifuging and washing three times in deionized water, the material is stained with safranin-O, mixed with glycerin jelly, and mounted on microscope slides.

Calibrated *Lycopodium* tablets

This is a standard approach by adding marker spores so that the concentration of pollen per gram of honey can be determined. *Lycopodium clavatum*, a fern ally, is a taxon of spores not collected by bees. Spores are acetolyzed, stained, and pressed into tablets. Lund University produces these tablets and determined that Batch 3862 averages 19332 spores per 2 tablets (standard deviation 4.91%). The formula to determine number of pollen per gram of honey is

$$(\# \text{ of pollen grains counted}) / (\# \text{ of } Lycopodium \text{ added}) / (\# \text{ of } Lycopodium \text{ counted})$$

We followed Jones & Bryant in counting 200 pollen grains for the concentration analysis, if that many could be found. Table 1 shows results of the pollen concentration.

Table 1. Total Pollen Concentration

	# <i>Lycopodium</i>	# Pollen Grains	Pollen per g honey
Big Box	200	0	0
Columbus County	195	200	19,800
Wake County	21	200	92,000

Table 2. Preliminary Pollen Abundance by Morphological Category (Pollen Sum =100)

	Monoporate	Triplicate	Multiporate	Monocolpate	Tricolpate	Tricolporate	Indet.
Columbus Co.	0	29	0	5	24	42	0
Wake Co.	1	0	25	15	7	40	12

Pollen Flora

Taxonomic analysis of the pollen flora of the honeys is at a preliminary stage (Table 2). We have identified *Liquidambar* (sweet gum; multiporate) and *Trifolium* (clover) in these honeys, but it will take further work to distinguish the abundant tricolporate forms. Jones & Bryant identified some taxa you might not initially expect in their honeys from East Texas (e.g., *Rhus toxicodendron* aka poison ivy); we will have to examine ours with care to see if similar results apply in North Carolina.

Table 3. Taste Test	
Honey	Percent Preferred
Big Box Retailer	10%
Columbus County	50%
Wake County	40%

Table 4. Taste Purity	
Honey	Percent Preferred
Big Box Retailer	6%
Columbus County	50%
Wake County	44%

Tables 3 and 4. Results of tasting panel. Table 3 is the taste preference, whereas Table 4 is the taste panel's evaluation of purity of taste. Total of 14 taste panelists.

Table 5. Physical Test Results and Qualitative Honey Analysis

Characteristics	Big Box	Columbus County	Wake County
Color	Light	Amber	Light Amber
Visual Observation	Air bubbles	Bubbles, hairs	Bubbles, bee parts
Texture	Runny	Sticky	Thick
Taste	Sweet/not natural	Sweet/extra sweet	Sweet
Purity	No	Yes	Yes
Flame Test	Not at all	Small flame	Burned readily



Figure 3. Microscope Fields of View of each honey sample to show relative pollen abundance. Note that the Big Box honey (Sample A) contains only *Lycopodium* markers and no pollen. B= Columbus County; C= Wake County.

Discussion and Conclusions

The "Tests for Basic Physical Characteristics" and taste tests (Tables 3-5) resulted in agreement that the Big Box honey seems less "pure" (including probably higher in water content) and tastes inferior to the North Carolina honeys.

Pollen Concentration. Qualitative abundance of pollen in each of these honeys can be seen in Figure 3. Quantitative concentration results are in Table 1. The Big Box Retailer honey has been ultra-filtered to remove the pollen (and other organic debris common in honey). There are many reasons to do this. One common reason is to obscure the origin of the honey because the pollen can easily indicate if the honey was imported and from where. The Columbus County honey pollen concentration is considered "very poor" on a standard scale despite the thousands of pollen per g, whereas the Wake County honey is high "intermediate" (Jones & Bryant, 2014).

Additional work will be needed to identify pollen taxa and delineate differences among honeys more specifically. We further expect to build a library of local to regional honeys for comparative analysis.

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References

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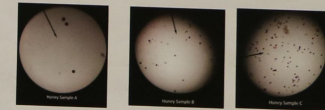


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