Debunking the Rumoured Water Test for Honey Purity Testing

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Abstract

Honey fraud is a major threat to the global honey market. Because of this, most honey sold in Canada is tested in laboratories using complex technologies such as DNA barcoding. But for people at home without access to professional laboratories, online websites provide DIY tests that claim to detect honey adulteration. One of these online tests called "the water test" claims to be able to detect honey adulteration using just water. The water test claims that adulterated honey, unlike pure honey, will dissolve in water upon stirring. Our study aims to assess the authenticity of the water test, by examining whether it can detect water and corn syrup adulteration in honey samples. We hypothesised that the honey test will be able to detect honey adulterated with corn syrup and water. To test our hypothesis, we had 4 treatments: pure honey, pure corn syrup, honey adulterated with water and honey adulterated with corn syrup. We stirred the 15 grams of the treatment into water and weighed the samples afterwards to see if it dissolved. We found that after performing the test, the pure honey sample lost 0.016% of its weight, the pure corn syrup sample lost 7.97%, the honey and corn syrup sample lost 5.00%, and the honey and water sample lost 63.75% of its weight. After running the Kruskal-Wallis test and Tukey multiple comparisons test, we found that the water test could strongly detect honey that was adulterated with water but struggled to detect honey that was adulterated with corn syrup.

Introduction

Honey is known for its many benefits including chemopreventive properties (Saiful Yazan et al., 1), anti-inflammatory, antibacterial, high antioxidant capacity, and free radical-scavenging activity (Kassim et al., 1). Honey's many benefits and taste has raised its commercial demand, and have become a major target for adulteration (Jaafar et al., 2). There are two prominent ways that honey can be adulterated, either through indirectly overfeeding bees and the overuse of drugs, or directly adding water and foreign sugars (Jaafar et al., 2). The addition of adulterants decreases the benefits of honey, changes the taste, and results in adverse health effects based on the type of adulterant (Fakhlaei et al. 2). Therefore, it is important for consumers to be able to verify the pureness of purchased honey.

A DIY water test found online ("How to Check if Your Honey is Pure or Adulterated") claims that adulterated honey would dissolve in water faster than pure honey. The validity of online tests are important as false tests mislead consumers into making unwanted decisions; if the water test was not trustworthy, then consumers would ingest adulterants which may affect their health. For example, excessive fructose intake can damage liver function and increase insulin resistance (Novelle et al., 1), and glucose can cause elevated blood sugar levels. To debunk this test, we investigated if it could detect crystallized honey that has been adulterated with water or high fructose corn syrup (HFCS) by comparing the dissolution rates of pure and adulterated honey. We chose adulterants that had lower viscosity than crystallized honey because with high viscosity, it is more difficult for molecules to move and thus results in a lower diffusion rate. Therefore, adding adulterants of lower viscosity to crystallized honey will lower the viscosity and yield "honey" that has a faster dissolution rate. For our experiment, water was chosen since it will dilute the nutritional value of honey and HFCS containing glucose and fructose was used since the extra sugar can cause adverse health effects. When consumed at moderate amounts, HFCS does not cause noticeable health effects (Khorshidian et al), but when mixed into honey and not clearly labeled, consumers risk over-ingestion of sugar.

In this experiment, we calculated the percent change of sample honey weight after it was stirred in room temperature water. Because HFCS and water are both less viscous than honey, we predicted that if we diluted crystalized honey using HFCS or water, then the diluted honey would dissolve into water faster. Our null hypothesis for statistical analysis states that there are no differences in the dissolution rates of all samples. On the other hand, the alternative hypothesis states that there will be differences in the dissolution rate of all samples.

Methods

To test whether or not the water test could effectively catch the water and corn syrup adulteration of honey, our experiment had a total of four treatments: the first and second treatments were the controls of the experiments, they were 100% honey and 100% corn syrup respectively, the third treatment was 76% honey/ 24% water, and the fourth treatment was 76% honey/ 24% corn syrup. We mixed our honey with a 24% volume substitution of corn syrup or water to mimic the adulteration process.

Each group member conducted their own independent trial, for a total of 4 trials. For their respective trial, each group member performed one replicate of all four treatments. Trials were conducted in different locations in the Lower Mainland of British Columbia. The spoons, cups, and electronic weighs used by individual members differed. The scales we used differed in resolution, two of the scales used measured to the closest gram, while the other two measured to the closest tenth of a gram. Ingredients used for the treatments were standardized. For the honey, PC brand organic honey was used. For the corn syrup, we used noname brand corn syrup. And for water, we used tap water which was left to reach room temperature (about 25°C).

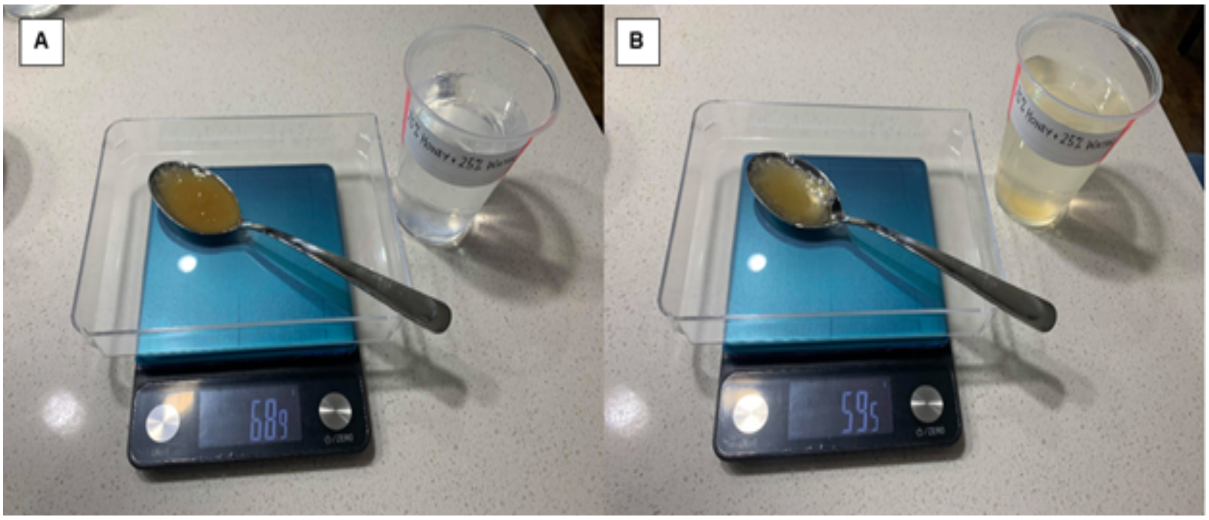

Figure 1. Image taken from a 76% honey/24% water replicate performed in Vancouver on March 30th, 2021. (a) The before picture -- the spoon and 15 grams of the 76% honey/24% water weighed 68.9 grams. (b) After the spoon and honey mixture was stirred in the water, the mixture sample lost 9.4 grams in weight.

To perform the water test, each member measured roughly 15 grams of their treatment mixture onto a tablespoon. We recorded the initial weight of the honey mixture alone and then we measured the weights of the spoon and honey mixture together. The spoon with the mixture was then stirred horizontally in a cup of room temperature water ten times so that the sample would not slide off the spoon. The spoon was shaken several times to remove excess water and then weighed to see how much of the mixture was dissolved in the water. The process was repeated by each member for all four treatments (Figure 1).

$$\frac{\textbf{Weight of honey sample alone} - (\textbf{Weight of spoon with sample before test} - \textbf{Weight of spoon with sample after test})}{\textbf{Weight of honey sample alone}} = \textbf{Percent change}$$

Figure 2. The formula we used to determine the percent change in the samples before and after stirring in a cup of water 20 times. We rounded the percent change to the nearest tenth of a decimal.

We recorded our data with the before and after weights of the mixtures with the weight of the spoons subtracted and calculated the percent change to the nearest 0.1 decimal (Figure 2). To analyze the data, we used Graphpad Prism. The assumptions of the one way ANOVA test were not met, thus we conducted a non parametric Kruskall-Wallis test. Further, we conducted a Tukey multiple comparisons test to determine where the differences were specifically between the different treatments.

Results

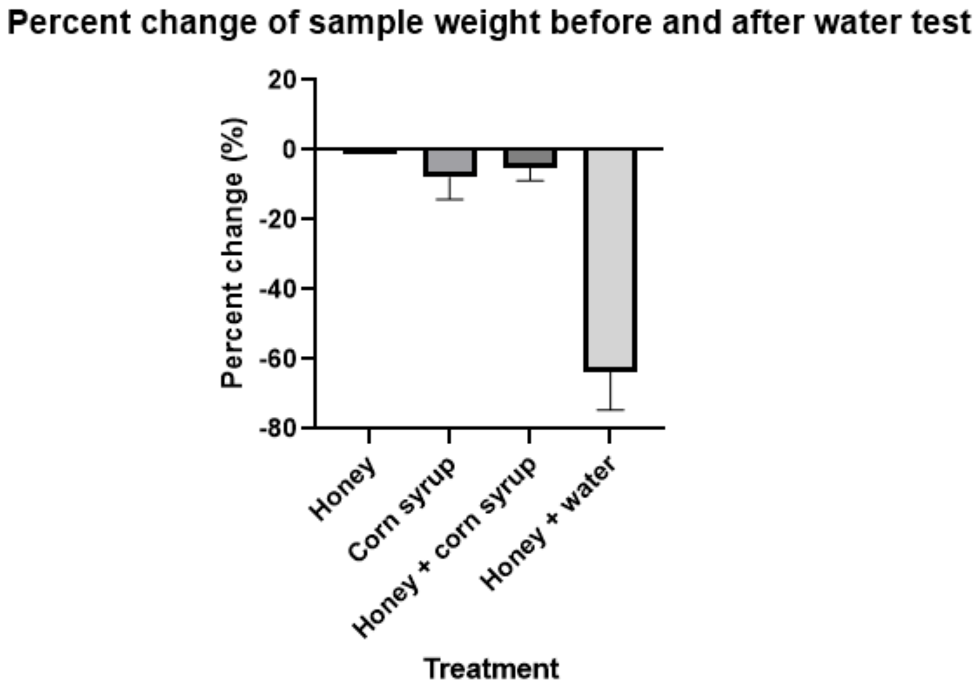

Figure 3. The mean percent change for pure honey, corn syrup, honey with corn syrup, and honey with water after the water test was +0.16%, -7.97%, -5.00%, and -63.75% respectively. Error bars represent the standard deviations (SD). SD for honey, corn syrup, honey with corn syrup, and honey with water are 0.33, 6.21, 3.79, and 10.91 respectively. The n= 4 and trials = 4.

The mean percent changes varied for each treatment type. The percent change for the pure honey sample (+0.16%) was the smallest, followed by the honey with corn syrup (-5.00%), and then the corn syrup (-7.97%) sample. The percent change for honey with water was the largest (-63.75%) (Figure 2.). The increased percent change for the pure honey sample after the water test was due to additional water weight. The honey mixed with water was more likely to dissolve into the water bath and pure unadulterated honey did not dissolve into the water at all. The honey with water sample had the largest standard deviation (10.91), the corn syrup sample had the second largest standard deviation (6.21), the honey with corn syrup sample had the third largest standard deviation, and the honey sample had the smallest standard deviation (Figure 3). Each standard deviation bar does not overlap in Figure 3. An alpha value of 0.05 was used for the non parametric Kruskal-Wallis test. The value of alpha was based on previous studies that detected adulteration in honey (Dramićanin et al., 3). The P value for our results was 0.0011. Thus, $P < 0.05$. Furthermore, the Tukey multiple comparisons test indicated that the differences between the mean percent changes exist only between the honey and the honey with water treatment. The adjusted P value for the difference between the honey and honey with water treatment was 0.0062.

Discussions

The data we collected allow us to determine if the test accurately indicated adulteration in honey by showing us on average, how much honey sample was lost or gained due to water weight after conducting the water test. The $P< 0.05$ for our results (0.0011) indicates that our results are significantly different from each other. In addition, the results from our Tukey multiple comparisons test indicate that the honey test can successfully determine if a sample has been adulterated with at least 24% water because $P=0.0062$ which is less than our alpha value. A P less than alpha (0.05) indicates statistical significance. Analysis of our data showed no significant differences between the other samples tested, hence it was determined that the water test was only able to detect water contamination.

Our alternative hypothesis states that we expect the water test to be able to detect honey adulteration when honey is mixed with either corn syrup or water. Therefore, the percent change in the weight of honey samples adulterated with water and corn syrup before and after the water test would be statistically different from the percent change in weight of the pure honey sample before and after the water test. However, we only saw statistical differences between the percent change in weights when honey was adulterated with 24% water. As a result, we reject our null hypothesis because our P value was lower than alpha (0.05) and the Tukey's multiple comparisons test also indicated that the percent change in weight for the honey sample mixed with water was significantly different from the percent change in weight for the pure honey sample.

The unexpected corn syrup results might be due to experimental errors that are explained below. Furthermore, we surmise that although corn syrup is visibly less viscous than crystallized honey and has a relatively lower dissolution rate, the dissolution rate might be so close to honey that we cannot see significant changes without more trials and longer stirring time. A follow up experiment that measures the viscosity of all samples and calculates their dissolution rates should be conducted to verify this surmise.

Furthermore, a study conducted by Abdel-Aal et al. shows results that are consistent with the results that we have found in our study. The study states that detecting corn syrup in adulterated honey was difficult. The sugar content found in honey and honey adulterated with 20-30% corn syrup was so similar that specified equipment was required to detect the differences. Furthermore, corn syrup adulterated honey is very similar in physical properties to natural honey. (Kelly et al., 2)

Limitations to our study includes variability in the precision of measuring tools and the shapes and sizes of our spoons. Our data was collected by 4 different people in 4 different kitchens using 4 different sets of tools. Our measuring scales were different and only some scales were able to detect decimal places. This created room for error in precision of the honey sample weights. Variability in the shape of our spoons may have also created error in our study since the surface area of the solvent that was in contact with the solute will affect the dissolution rate. In addition, the speed and force at which each experimenter stirred their spoon was a challenge to control for given that each experimenter was in separate kitchens when conducting their trial of the experiment. Therefore, some of the differences we see across the trials might be due to how strongly the experimenter was stirring their spoon. It was also hard to keep all materials at 25 degrees Celsius in a room with uncontrolled temperature. The difference in temperature in the honey, water, and corn syrup could have influenced how well the material would dissolve in the water. We suggest replicating this experiment in the future in a temperature controlled lab with specialized equipment. In addition, utilizing a larger sample size will lead to more representative results.

In terms of practical implementation, this study demonstrates that the water test is not 100% accurate for honey adulterated with corn syrup. If a consumer relied on the water test, then they would likely eat much more fructose and/or glucose than they intend to and be susceptible to potential health risks (Novelle et al., 1). Further studies should consider the different types of adulteration that happens in the honey business, other online tests that have not been scientifically verified and different types of honey (e.g. liquid or creamed).

Conclusion

The goal of this study was to test whether the water test was a reliable way to determine the purity of a honey sample by adulterating our own honey sample with water or corn syrup. The results indicated that there is a significant difference in the amount of honey weight before and after the water test between samples of pure honey and samples of honey with water only. The Tukey test did not determine any significant difference for other samples. Thus we reject the null hypothesis because we do see a difference in the amount of honey dissolved in water after the water test and conclude that the water test can detect honey that has been adulterated with at least 24% water content by volume. However, we fail to determine if the water test can identify honey that has been adulterated with corn syrup with at most 24% by volume. Therefore, the water test is best at determining the purity of a honey sample when it has been adulterated with water and should be used with doubt for honey contaminated with corn syrup.

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Appendix

Table 1. Raw data for honey dissolving in water. Percent change calculated as ((weight after - spoon weight) - (weight before - spoon weight)) / (weight before - spoon weight)

	Weight before	Weight after	spoon weight	Percent change
Honey 1	64.4	64.5	49.0	+0.65
Honey 2	68.9	68.9	53.9	0%
Honey 3	20	20	5	0
Honey 4	15	15	0	0
Corn Syrup 1	56.7	56.4	41.3	-1.9
Corn Syrup 2	68.9	68.4	53.9	-3.3%
Corn Syrup 3	20	18	5	-13.33
Corn Syrup 4	15	13	0	-13.33
Honey + Corn Syrup 1	64.2	64.3	49.0	+0.66
Honey + Corn Syrup 2	68.9	67.8	53.9	-7.33
Honey + Corn Syrup 3	20	19	5	-6.67
Honey + Corn Syrup 4	15	14	0	6-.67
Honey + Water1	56.3	44.4	41.3	-79
Honey + Water 2	68.9	59.5	53.9	-62.67%
Honey + Water 3	20	11	5	-60
Honey + Water 4	15	7	0	-53.33

Table 2. Kruskal-Wallis statistical analysis test results

Kruskall - Wallis test	P value (exact)
All 4 groups	0.0011

Table 3. Tukey's multiple comparisons test results.

Tukey's multiple comparisons test	Significant?	Summar	Adjusted P value
Honey vs. corn syrup	No	ns	0.6048
Honey vs. honey + corn syrup	No	ns	>0.9999
Honey vs. honey + water	Yes	**	0.0062
Corn syrup vs. Honey + corn syrup	No	ns	>0.9999
Corn syrup vs. Honey + water	No	ns	0.6048
Honey + corn syrup vs Honey + water	No	ns	0.1514