

## Modified Equations to Calculate Water Content and Refractive Index of Honey Based on Its Total Soluble Solids

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**Abstract:** The capacity of honey to break light is used for refractometric determination of humidity. In the present work, a manual refractometer was used to measure honey total soluble solids (73% - 86%) which are the corresponding values of refractive indices stated in Chataway's Table. The relationship between values of total soluble solids, water content and refractive index in the obtained graphics gave equations that could be used directly to calculate both refractive index and water content of honey from its total soluble solids. This simple, fast and easy way could be applied without utilizing expensive refractometers due to their high cost or lack of their usage training. Furthermore, the suggested equation is more accurate than that of the current reference table (i.e. Chataway's Table) and could be rapidly utilized for calculating water content in honey samples.

**Keywords:** Modified equation; Honey; Moisture content; Refractive index; Total soluble solid.

### 1. Introduction

The physical characteristics of honey are important criteria in honey harvest, processing technology, granulation, storage and liquefaction [1-4]. The water content is a vital quality parameter which affects the honey shelf life [5,6]. The over-grade water content causes fermentation that spoils aroma and flavor of the honey. Honey water content is also closely associated with yeast count, and very minimal fermentation degree was recorded in the honey containing 17% humidity due to the very low yeast count [1,2]. The measurement of honey moisture is quality criterion to determine the stability of honey, and its resistance to yeast fermentation. According to international standards, the estimation of water content of honey is the most important physical factor used to evaluate honey's quality [7-9].

The moisture content in honey is directly associated with its botanical origin, environmental conditions (temperature and relative humidity) during production of honey, harvesting season, processing and storage situations [10-13]. Honey has ability to break the light, and this ability is used for the determination of humidity with refractometer in terms of refractive index (RI) [14]. The data of RI represent the proportion of water in honey, which is often calculated using a standard table depending on RI of honey measured by manual Abbe's or digital refractometers [15]. The table is based on a formula [Water content (%) in 100 g honey =  $1.73190 - \log (RI-1) / 0.002243$ ] developed by Wedmore [16] from the data given by Chataway [17].

This formula is very complicated and it could be replaced by new simple one by plotting the relationship between refractive index and water content of the same values recorded in the same table. Moreover, the selection of the most suitable methods depends on whether the sample is in solid or liquid forms, time consumed, and the available equipment.

The aim of this work is to summarize Chataway's Table [17] in a simple equation which could be used directly for calculating both of refractive index and water content of honey to the nearest decimal number. The measuring devices (Abbe's refractometer) is expensive, not usually available and needs training compared to portable devices (Zeiss refractometers or hydrometers) which are simple, cheap and available in most laboratories.

## 2. Materials and Methods

### 2.1 Honey samples

The samples of honey were taken from stock honey jar, weighed (5g each) and diluted with distilled water (1g honey: 9ml water). The cabinet of an Abbe's refractometer was filled with diluted honey and the readings of %TSS (total soluble solids) were recorded.

### 2.2 Modification in equation

The original equation is developed by Wedmore [16] from the data of Chataway [17] as given below:

$$W = 1.73190 - \log(RI - 1) / 0.002243$$

where W is the water content (g) in 100 g honey and RI is the refractive index.

A refractometer (Carl Zeiss, Jena) having two scales for measuring refractive index (from 1.3000 to 1.7000) and TSS (from 0% to 95%) was adjusted at 20° C. TSS values (72% to 86%) matching refractive indices (RI) were measured as stated in the Chataway's Table at the same base line present in the refractometer (1.3330 & 1.5320 RI matching 0% & 95% TSS, respectively).

The concentration of TSS was corrected at 20° C using the following formula:

$$\text{Water Content (WC) at } 20^{\circ}\text{C (\%)} = (1.5365 - RI) / 0.0025$$

(If temperature exceeded 20° C, 0.00023 was added per 1° C and when it was below 20° C: 0.00023 per 1° C was subtracted).

$$\text{TSS (\%)} = 382.96 (RI) - 490.92 \text{ (Figure 1)}$$

$$\text{Refractive index (RI)} = \text{Total soluble solids (TSS\%)} + 490.92 / 382.96 \text{ (Figure 1)}$$

$$\text{WC (\%)} = (1.5365 - RI) / 0.0025 \text{ (Add 0.12 if } RI \geq 1.4982)$$

$$\text{WC (\%)} = -396.14 (RI) + 608.88 \text{ (Figure 2)}$$

$$\text{WC (\%)} = -1.034 (\text{TSS\%}) + 101.02 \text{ (Figure 3)}$$

### 2.3 Statistical Analysis

A statistical measure R-squared (R<sup>2</sup>) and regression line equations (Y) were calculated by applying linear regression analysis with manually designed equations between different variables (TSS vs WC, TSS vs RI and WC vs RI). A regression line showed the relation between the dependent "y" variable and independent "x" variables when there is a linear pattern. The data were statistically analyzed using Excel Program.

## 3. Results

The data illustrated that the simple modified formulas could be used directly to calculate both of refractive index (RI) and water content (WC) in honey. Figure 1 showed the relationship between RI and TSS of honey. TSS and RI has positive direct increasing regression trend. Greater the TSS value corresponds to higher RI value of the honey and vice versa. The linear regression R<sup>2</sup> values were 0.999 and linear equation was  $y = 382.96x - 490.92$  (Figure 1).

The Figure 2 showed the relationship between WC and RI of honey at 20.0°C. The values of RI showed inverse relationship to WC. Higher value of WC corresponds to the lower value of RI and vice versa. The linear regression R<sup>2</sup> value was 0.999 and linear equation was  $y = -396.14x + 608.88$  (Figure 2).

The Figure 3 showed the relationship between WC and TSS of honey at 20.0°C. The values of TSS showed inverse relationship to WC. Higher the value of WC corresponds to the lower value of TSS and vice versa. The linear regression R<sup>2</sup> value was 0.999 and linear equation was  $y = -1.034x + 101.02$  (Figure 3). In conclusion, WC has direct positive relationship to RI but inverse negative indirect relationship to TSS and RI.

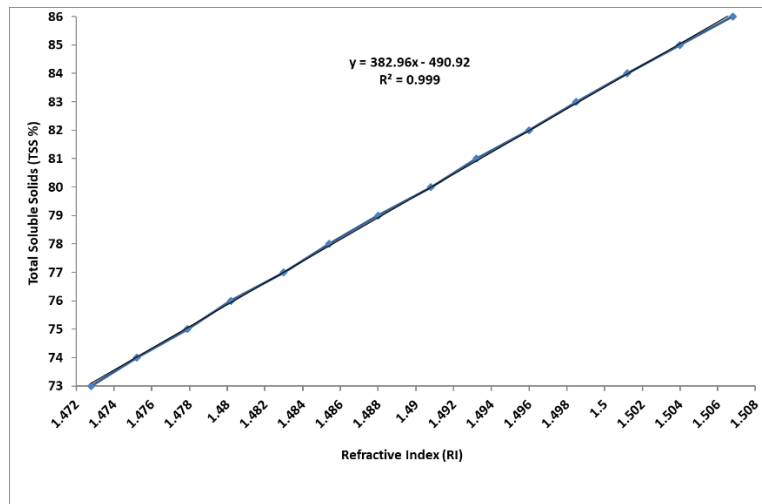


Figure 1. Relationship between Refractive index (RI) and Total Soluble Solids (TSS) at 20 °C using the formula:  $[RI = TSS (\%) + 490.92 / 382.96]$

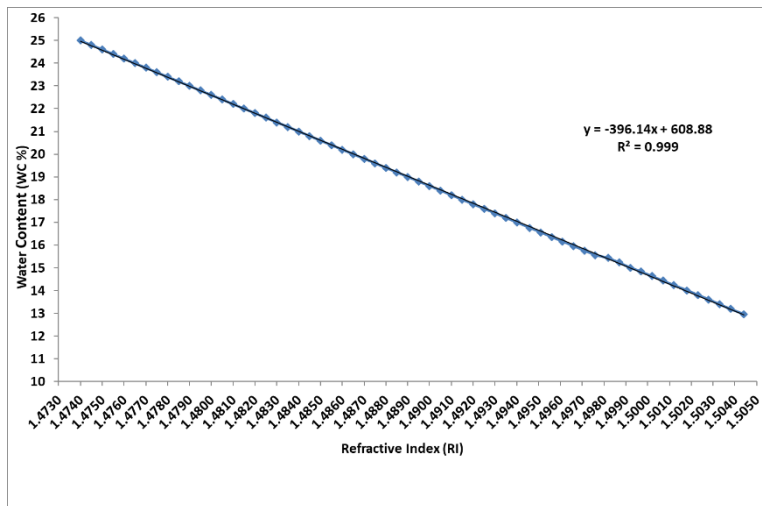


Figure 2. Relationship between Water content (WC) and Refractive index (RI) at 20 °C using the formula:  $[WC (\%) = -396.14 (RI) + 608.88]$

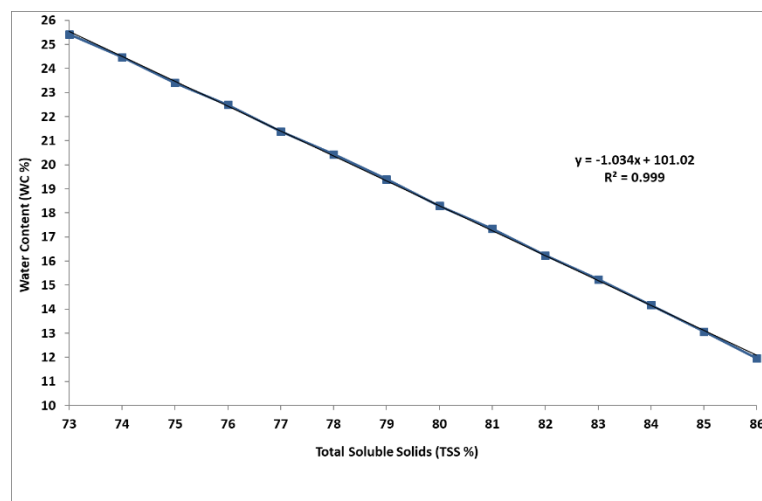


Figure 3. Relationship between Water content (WC) and Total soluble solids (TSS) at 20 °C using the formula:  $[WC (\%) = -1.034 (TSS\%) + 101.02]$

## 4. Discussion

Although, both manual and digital refractometers can be used, their measurement does not reflect the accurate water content value in honey. Indeed, measurements of water content by Karl-Fischer (KF) method (a more complicated and expensive technique) shows that the refractometric measurements overestimates the true water content by about 1 Brix unit [18,19]. Besides, in some cases the values of refractive index (RI) are not included in the table. Therefore, water content (WC) requires consequent calculations to be estimated. Contrarily, the present method can directly estimate any WC in honey using total soluble solids regardless of RI value.

Two official methods are used to assess water content; the gravimetric determination after oven drying and the RI method with an evaluation of moisture percentage using empirical formula or relative conversion table [20]. RI determination is the most commonly used method. However, the difficulty in this technique is that the empirical formula or relative conversion table used for evaluation of moisture content is not likewise correct for every type of honey.

The moisture content is one of the most prevalent determined characteristics of foods for numerous reasons, e.g. legal and labeling requirements, microbial stability, food quality and food processing [21]. In addition, some physical properties such as viscosity and crystallization are affected by moisture content of honey [12,22]. Different analytical techniques have been developed for this purpose with variations in accuracy, cost, speed, sensitivity, specificity, and ease of operation depending on the nature of the material being analyzed. These techniques include evaporation and distillation, physical, spectroscopic, thermogravimetric, calorimetric, and chemical reaction (gas production), and Karl-Fischer method [23,24]. KF method is based on titration which exploits a selective chemical reaction: the oxidation of sulfur dioxide by iodine in the presence of water. The change in color when water reacts with the added chemicals is then measured. Since, the sulfur dioxide and iodine are gaseous and normally being lost from solution, this reaction has been modified by adding certain solvents (e.g. pyridine). The sugars are not much soluble in this medium and honey dissolution is slow, a possible solution is the use of warm methanol in a titration vessel with thermostatic jacket [22]. Although the precision of KF titration can be improved by spectrophotometric detection, certain compounds, e.g. ketones and aldehydes interfere with basic KF titration, that react with methanol to form water, which causing incorrectly (high) water values, and vanishing endpoints. Moreover, specialized laboratories and costly devices are needed [25]. Furthermore, value of water content obtained by KF method is usually higher than that determined by RI analysis [26]. Although it was indicated previously that RI and KF methods resulted similar moisture values in honey, mainly when methanol and formamide were used in KF [24]. However, the same authors mentioned that moisture determination in honeys by refractometry does not yield the “true” moisture content. They attributed the variation in composition of soluble solids of different honeys which affect the conversion of RI into moisture content.

## 5. Conclusion

The present modified equation could be directly used to calculate the refractive index and water content of honey from its total soluble solids measured by manual refractometer. It is simple, fast and easy way, and doesn't need expensive refractometers. The proposed equations gave accurate values compared to Chataway's table.

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