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Brief Communication

Literature review on linear regression equations for relating water activity to moisture content in floral honeys: Development of a weighted average equation

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Abstract

A literature review on several existing linear regression equations for correlating water activity (a_w) and refractometric moisture content in floral honeys was performed in order to provide a weighted average regression equation. For this purpose, a meta analysis of the literature linear equations was made which takes into account the number of data points as well as the precision involved in the different literature studies. The weighted average linear regression equation is a general linear relationship to calculate the honey water activity on the basis of the honey moisture content. It was obtained from comparing and combining the results of ten independent studies involving 638 observations and including honeys from Argentina, Colombia, Spain, Germany, Czech Republic, China, México, Cuba, Brazil, El Salvador, India and Vietnam. It was also found that the proposed equation describes satisfactorily the water activity of concentrated (and supersaturated) glucose / fructose solutions, which would confirm that these sugars are the main determinants of water activity in honey.

Key words : Honey; Water activity; Meta-analysis; Regression; Moisture Content

Introduction

It is well known that honey fermentation is controlled by its water activity (a_w) rather than the moisture content. Knowledge of water activity of honey is also needed to predict moisture exchange with the environment, since water activity ($a_w = p/p_o$, where p is the water vapor pressure in honey, and p_o the water vapor pressure of pure water) is the difference behind water transfer from/to honey (Zamora *et al.*, 2006; Chirife *et al.*, 2006). However, honey industry utilizes almost exclusively the moisture content (determined by refractometry) as a criterion of microbial stability in honey. For this reason many researchers tried to find a relationship between water activity and refractometric moisture content (M %) in floral honeys, which takes a simple form:

$$a_w = a + b \cdot M \% , \quad \text{eqn. (1)}$$

which adequately describes the moisture sorption isotherm over the range of 14-22 % moisture (Zamora *et al.*, 2006). Although equations proposed by different researchers were similar they were not identical, and this may be attributed to, a) sampling error , b) lack of accurate measurement of water activity (Chirife *et al.*, 2006), and c) variation in sugar composition among honey of different origins, as will be discussed later. Chirife *et al.* (2006) theoretically showed that for small moisture content intervals of honey, a linear relationship between water activity and % moisture should be expected. Several literature studies using floral honey from different origins have reported (Cavia *et al.*, 2004; Chirife *et al.*, 2006; Estupiñan *et al.*, 1998) such linear regression relationship; however, slope and

intercepts were not identical, but similar, as will be observed later in this paper. Coefficients of determination also varied from one study to another.

Sugars represent the largest portion of honey composition and the monosaccharides fructose and glucose are the most abundant, while small amounts of sucrose, maltose, and higher order sugars (trisaccharides and oligosaccharides) are also present in small quantities. Zamora *et al.* (2006) analyzed literature data on sugar composition of a very large number of floral honeys from USA, Italy, Spain, Australia, Saudi Arabia and several other European countries, and found that fructose and glucose represented nearly more than 80 % of total honey solids (range between about 79-93 %). On the basis of their molal concentration, fructose and glucose were the main determinants of water activity, while maltose and sucrose (or other disaccharides) were much less important. Since fructose and glucose have an identical effect in the honey water activity, and fructose and glucose account for more than 80% of the total solids in honey, then it is reasonable to assume that honey from different origins may have similar linear relationship between water activity and moisture content (Zamora *et al.*, 2006).

The purpose of the present study was to develop a general lineal relationship to calculate water activity in honey by taking a weighted average of previous linear relationships proposed in independent studies. For this purpose a meta-analysis of the reported equations was performed; meta-analysis is a technique that offers formal statistical methods for comparing and integrating the results of multiple studies (Hedges and Olkin, 1985).

Materials and Methods

Ten studies reporting linear relationships for correlating water activity and % moisture content in honey were selected from the literature. Practically, all water activity data were obtained at 20-25 °C. However, it is to be noted that water activity changes very little for moderate temperature intervals. Floral honey is derived from the nectar of many type of flowers while honeydew honey is derived from the sweet secretions of aphids or other plant sap-sucking insects. Since almost available literature data on water activity/moisture corresponded to floral honeys, we selected this type of honey. Also, the sugar profile of honeydew honey may be different to that of floral honeys. To be included in the present selection, literature data had to fulfill with the following criteria: a) honeys of floral type, b) number of honey samples utilized > 10; c) coefficient of determination (R^2) > 0.65, and, d) range of moisture contents, approx. 14-22 % w.b. In six of the selected studies, original data for water activity and moisture content were available, while in four of the studies only the intercept, slope and determination coefficient (or correlation coefficient) were informed. None of the ten studies reported standard error (SE) for both intercept and slope and therefore these were calculated from the original data or estimated using the mathematical association among slope, determination coefficient and sample size.

Data Analysis

I^2 statistic index was calculated in order to determine the degree of inconsistency across studies in a meta-analysis (Higgins *et al.*, 2003). This describes the percentage of the variability that is due to heterogeneity rather than sampling error. A value greater than 50% may be considered substantial heterogeneity. We found an I^2 statistic index of 60 and 67% for the intercept and slope respectively, suggesting heterogeneity between studies. This heterogeneity was due mainly to data from Gleiter *et al.* (2006). In fact, when these data were excluded from our analysis, I^2 statistic index lowered to 27 and 38% for the intercept and slope respectively. We decided not exclude these data to use the more conservative random effect model to pool studies. This model was used instead of fixed effect model, because it takes into account both between-study and within-study variability.

Pooled intercept and slope were obtained using weighted least squares approaches. This method has been previously used by Bini *et al.* (2001), who cited Hedges and Olkin (1985) as the basis of their approach. Following this method, the individual study results were weighted using inverse variance weighting. Thus, the overall weighted regression coefficient b (intercept or slope) was computed as

$$b = \frac{\sum_{i=1}^k w_i b_i}{\sum_{i=1}^k w_i}$$

where k is the number of studies combined, b_i is the regression coefficient from study i and w_i is the weight for that regression coefficient in the i th study. This weighting factor is the reciprocal of the sum of regression coefficient variance plus between-study variance τ^2

$$w_i = \frac{1}{V_{(b_i)} + \tau^2}$$

The variance of b is given as

$$V_{(b)} = \frac{1}{\sum_{i=1}^k w_i}$$

Results and Discussion

Table 1 gives details of the literature studies included in this work. Six hundred and thirty eight observations from ten investigations including honeys from Argentina, Colombia, Spain, Germany, Czech Republic, China, México, Cuba, Brazil, El Salvador, India and Vietnam, were considered. Studies varied scarcely in the methodology and in the moisture range. A “piori”, one could simply determine the average of literature linear regression equations between water activity versus refractometric moisture content. However, the use of meta-analysis offers a quantitative synthesis of data from independent experiments taking into account the precision of the estimations, which depends upon heterogeneity and sample size. Additionally, heterogeneity between study results may be detected and incorporated to the model.

Table 2 shows intercept, slope and statistical parameters of selected literature relationships for water activity versus % moisture in floral honeys.

The I^2 statistic index, that describes the percentage of total variation across studies that is due to heterogeneity rather than chance, was 67% and 60% for the slope and the intercept of the straight line relationships, respectively. Thus, between-study variances were significantly ($p < 0.05$) greater than zero, indicating substantial variation in the equations.

This heterogeneity between estimates could be explained by a) the different composition of the honeys (as discussed above), and b) error in measurements either due to sampling method and /or accuracy of water activity measurement.

Because of heterogeneity between studies, results were pooled by using the random effect model.

Based on the ten unweighted regression coefficients, overall weighted regression coefficients were computed. Each estimated coefficients was weighted by the inverse of the residual variance component, defined as the random-effects variance plus the estimation variance for each study. The weighted average linear regression equation was,

$$a_w = 0.2686 + 0.01756 \cdot M \% \quad \text{eqn. (2)}$$

The 95% confidence interval for the intercept was (0.2674-0.2699) and for the slope was (0.0175 – 0.0176).

Equation (2) is proposed to estimate the water activity of floral honeys from knowledge of their refractometric moisture content.

In a previous paper Zamora et al. (2006) determined the water activity of very concentrated (and supersaturated) solutions of glucose, fructose and the mixture glucose + fructose. They found that solutions of either glucose, fructose or 1:1 mixtures had the same water activity provided they had the same mass concentration. This behavior has been also reported by other workers, among them Favetto *et al.* (1982) and Baeza *et al.*, (2008).

Figure 1 compares the proposed weighted average linear regression equation for floral honeys (eqn.2) with the experimental data of glucose / fructose solutions. It may be noted that moisture content for these sugar solutions (symbols in Fig.1) was calculated gravimetrically when preparing the solutions from pure glucose and fructose chemicals. A good fit of experimental data of glucose/fructose solutions to eqn 2 was observed as judge by the R^2 of 99.60% obtained. This finding would corroborate that fructose + glucose are the main determinants of honey water activity.

Conclusions

A literature weighted average linear regression equation was proposed to calculate the water activity of floral honeys on the basis of their moisture content. It was obtained from comparing and combining the results of ten independent studies involving 638 observations and including honeys from Argentina, Colombia, Spain, Germany, Czech Republic, China, México, Cuba, Brazil, El Salvador, India and Vietnam. The proposed equation also describes satisfactorily the water activity of concentrated (and supersaturated) glucose / fructose solutions, which would confirm that these sugars are the main determinants of water activity in honey.

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Legends for figures

Figure 1 : Comparison of proposed linear equation for floral honeys (eqn 2) with water activity data for glucose / fructose concentrated solutions (○) reported previously (Zamora *et al.*, 2006).