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Moisture content and water activity relations in honey: A Bayesian multilevel meta-analysis☆

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ABSTRACT

Research objectives were to investigate i) linear moisture content (m) - water activity (a_w) relations in honeys, ii) quantify variability due to geographical origin, iii) prediction of a_w from m using this quantified variability. Bayesian multilevel modeling was used to reach these objectives using thirty literature data sets. The rate of change in a_w as function of m (the slope) was identical per origin, whereas actual values of a_w at the same m (intercepts) differed. Geographic variation characterized by multilevel modeling (partial pooling) was quantified and compared to single-level modeling with all data pooled (complete pooling) and single-level modeling for each origin (no-pooling). Multilevel modeling results in an overall slope and intercept at the population level but also in individual intercepts per origin, hence variation is characterized at two levels. The obtained multilevel population parameters predicted almost exactly the relation between a_w and m of glucose-fructose solutions resembling honey, confirming that a_w-m relations in honeys are determined by glucose/fructose but not the actual a_w-values themselves. Multilevel modelling, a compromise between over- and underfitting, gives the best prediction of a_w of honeys from m including origin variability. The applied procedure is a blueprint to characterize variation in all types of food.

1. Introduction

Honey is a product that is known and used all over the world. The basic components of honey are water, glucose and fructose, but its composition is very complex and contains many other components in minor quantities: disaccharides like sucrose and maltose, salts, organic acids. Thus, from a chemical and physical perspective, one could consider it a highly concentrated sugar solution but non-sugar minor components do have an effect on product characteristics as well. Two general reviews on honey composition and characteristics can be found in Machado De-Melo et al. (2018) and Wang et al. (2023).

With a low enough water activity (a_w) honey is microbial stable. However, there is a critical limit of a_w around 0.6, above which especially yeasts can grow. Water activity is therefore a critical quality characteristic and many papers have established a statistical (linear) relationship between water activity a_w and moisture content m (usually expressed in g water/100 g honey). It is striking that such relationships appear to have the same slope but quite often a different intercept (Chen, 2019). Obviously, as natural products, honeys from different regions can be quite variable in composition and characteristics. In food science in general, it is important to take this variability into account in a quantitative way.

Chen (2019) analysed six available honey data sets using 'modern regression analysis' with categorical F-tests and ANOVA. Another modern upcoming statistical technique is multilevel modeling that allows to quantify variability according to categories or clusters of data. Continuing on the work of Chen (2019), it was investigated whether or not multilevel modeling can be applied to the a_w and m relationships found in literature. Moreover, the data set was extended from 6 to 30 cases. Multilevel modeling can be applied in the frequentist framework as well as in the Bayesian framework (the differences are briefly explained below).

While the current application is on honey data, the approach can be applied to many other data sets that are subject to variability. The paper therefore attempts to show the possibilities of multilevel modeling also in a broader sense.

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2. Background and objectives

2.1. Statistical approach

The two philosophies in statistics comprise the frequentist and the Bayesian approach. During the past 100 years, the frequentist approach has become the dominant one where probabilities are based upon frequencies of events. In the frequentist framework, parameters are considered as fixed (i.e, have no variability) while data are considered variable: they are considered to represent an overarching population of all possible data. It leads to null-hypothesis testing, p-values, ANOVA and confidence intervals. While there is nothing wrong with this approach, interpretation of frequentist statistical results is not that straightforward. It is not always realized that frequentist statements are about data as in Eq. 1, it is not about hypotheses or parameters.

$$P(D|H) \tag{1}$$

This is a so-called conditional probability statement: what is the probability to observe data D given a hypothesis H. It is not uncommon that statistical results obtained in the frequentist way are actually interpreted in the Bayesian way because that is more the natural way people tend to think. In the Bayesian philosophy, then, parameters are considered variable and data fixed (once obtained), and the resulting statements are about the probability of parameters (or hypotheses) given the data, as in Eq. 2:

$$P(H|D) \tag{2}$$

Parameters are considered variables that cannot be observed but can be inferred. In the Bayesian framework, a probability statement can be made about a parameter, for instance, that there is 95% probability to lie between some specified values. This is not possible in the frequentist approach because a parameter has no variability by definition. So, a frequentist confidence interval for a parameter is not a statement about probability but about how frequent a parameter will be in, or out, a certain interval if the experiment or observation is made an infinite number of times. To make that distinction, the equivalent of a confidence interval in the Bayesian framework is called a credible interval, or as recently suggested by McElreath (2020), a compatibility interval (i.e., compatible with the observed data and proposed hypothesis). Numerically, the results obtained in the frequentist and Bayesian way are usually close but interpretation is different. The reason it is called a Bayesian approach is because of the application of Bayes' theorem, as summarized in Eq. 3:

$$P(H|D) = \frac{P(D|H)\cdot(P(H)}{P(D)} \tag{3}$$

This theorem makes it possible to convert probabilities about data $P(D|H)$ (where the frequentist approach leaves it) into probabilities of hypotheses or parameters $P(H|D)$ given the data. $P(H|D)$ is called the posterior probability. $P(H)$ is called the prior probability (the expectation for a parameter or hypothesis before the data are taken into account). $P(D)$, the likelihood averaged over the parameters and weighted by their priors, is just a number.

The Bayesian approach expresses uncertainty quantitatively in the form of probability distributions for parameters or hypotheses (the narrower, the more certainty), whereas the frequentist approach results only in point estimates of parameters. Though the frequentist way has become the dominant approach the past 100 years, the Bayesian approach is actually older, but computationally more complex and could therefore, until recently, only be applied to relatively simple problems. The computational problem is in $P(D)$ that can contain complex integrals. But with the advent of computer power this problem can be circumvented by sampling and very complex modeling problems can now be approached in the Bayesian way via Markov Chain Monte Carlo (MCMC) techniques. The reader interested in how MCMC works is referred to background literature (e.g., McElreath, 2020; Lambert, 2018; Kruschke, 2015; Gelman et al., 2013). Software for MCMC calculations is available; the current state-of-the-art software is called Stan (Gelman et al., 2015; Carpenter et al. 2017) and is used in this paper.

Important to know is that in the Bayesian approach a likelihood function ($P(D|H)$ in Eq. 3) and a prior probability statement about parameters ($P(H)$ in Eq. 3) need to be specified by the researcher in order to be able to apply Bayes' theorem. This practice forces scientists to state the assumptions in the open and make them debatable. Other researchers may have different opinions and may come to other conclusions with the same data. This is perfectly fine, though some people may argue that this makes the Bayesian approach subjective and consider that not scientific. However, assumptions are also made in the frequentist approach but they are often not stated. Without diving in this debate, it can be stated that the Bayesian approach has by now become a well-accepted approach that is used more and more in many branches of science. However, not so much yet in food science with a few exceptions (Van Boekel, 2020, 2021a, 2021b; Garre et al., 2020; Garre et al., 2022). The present paper is a further attempt to show how the Bayesian approach can be used in food science.

2.2. Multilevel modeling

Variability is a natural phenomenon when studying biological materials like foods. Instead of hiding variability by calculating averages (loss of information), it is important to quantify variability explicitly and multilevel modeling offers that opportunity. The Bayesian approach appears to be very suitable to use in this respect because it allows variability in parameters (though it is also attempted in the frequentist approach via likelihood methods). Modeling has basically two functions: i) to scientifically understand relations between variables, ii) to be able to make predictions. The two do not necessarily go together: it is perfectly feasible to make predictions for relations that are not understood but only established empirically by some mathematical relation. However, it is obvious that predictions based on scientific, mechanistic insight will be more reliable in principle, which is attempted in this paper. Being able to not only predict values of variables but also the uncertainty in those predicted values is of great practical significance.

What happens often with sampling in practice is that measurements or observations are somehow connected (are correlated in statistical terms). For instance, honeys collected from a certain region, of from a specific bee species, have something in common. However, standard regression procedures assume that data are independent and not connected. Multilevel modeling takes these dependencies into account and may therefore lead to better, unbiased estimates, as well as that it gives better insight in variability.

Perhaps, the principle is best illustrated, in relation to the current topic, with a simple schematic picture about variability of water activity in honey: see Fig. 1. Starting with common single-level modeling (Fig. 1A), there will be variation around a central value (like the mean or the median). The variation is quantified by residuals (difference between individual values and the mean) and summarized in a residual variance; this is the simplest model one can apply. Single level modeling can be turned into multilevel modeling if data can be grouped into clusters: see Figure1B. In the case of honey, clusters could arise based on a certain geographic origin. Now, one can calculate a mean and residuals for each cluster (first level) as well as an overall mean and residuals at the second level (difference between cluster means and the overall mean). The actual measurements are always at the first level, second and higher levels arise because of clustering. There can be more than two levels, obviously. For instance, for honeys, clustering could be done for types of bees, for different regions in a country, for different countries.

Referring to Fig. 1A, the variance obtained with single level modeling for n data with residuals r_i is calculated as in Eq. 4:

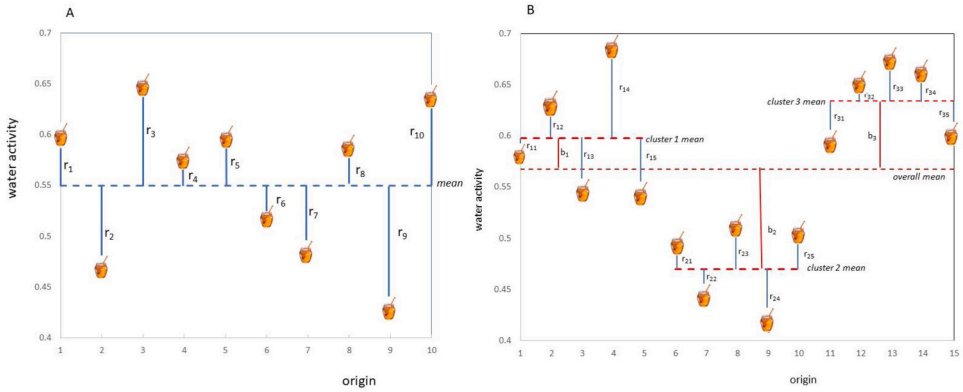

Fig. 1. A: Schematic example of single level model of i = 1..n honey samples (here: n = 10) with one type of residual (r_i). B: Multilevel model with i = 1..n samples (here: n=5) in j=1..k clusters (here: k=3) resulting in two types of residuals r_{ij} and b_j (after Sommet and Morselli, 2021).

$$\mathrm{var}(r_i) = \sigma_r^2 = \frac{r_1^2 + r_2^2 + \ldots r_i^2}{n} \tag{4}$$

In the case of two-level modeling (Fig. 1B) there are now two variances for i samples with residuals r_{ij} and j clusters with residuals b_j, as in Eq. 5:

$$\mathrm{var}(r_{ij}) = \sigma_r^2 = \frac{r_{11}^2 + r_{12}^2 \ldots + r_{21}^2 + r_{22}^2 + \ldots r_{ij}^2}{n} (i = 1..n, j = 1..k)$$
$$\mathrm{var}(b_j) = \sigma_b^2 = \frac{b_1^2 + b_2^2 + \ldots b_j^2}{k} (j = 1..k) \tag{5}$$

While Fig. 1 refers to a very simple modeling case with only means and variances, the principle can also be applied to more complicated linear and nonlinear models with intercepts and slopes and other parameters, as will be illustrated below.

Further introductions to the Bayesian approach as well as multilevel modeling can be found in Johnson et al. (2022) (also available as free e-book BayesRules!) and McElreath (2020).

2.3. Relation between water activity and moisture content

As indicated in the previous section, understanding the science behind relations is very helpful in establishing models. The relation between water activity and moisture content is of a thermodynamic nature, and therefore relates to equilibrium conditions. Water activity is defined as in Eq. 6:

$$a_w = \frac{p}{p_0} \tag{6}$$

where p is the pressure above an aqueous solution and p_0 the pressure of pure water. Clearly, $0 \le a_w \le 1$, so $a_w = 1$ means pure water is present and $a_w = 0$ means absence of water. Solutes will alter water activity and the relation cannot be predicted in theory except for ideal solutions (where no interactions occur between solutes and solvent and between solutes themselves). In an **ideal solution**, water activity (a_w^{id}) changes with the molality of a solute independent of its nature according to Eq. 7:

$$\ln a_w^{\mathrm{id}} = \ln X_w = \ln\left(\frac{1}{1 + m_i M_w}\right) = -\ln(1 + m_i M_w) = -M_w m_i \tag{7}$$

X_w represents the mole fraction of water, M_w the molar mass of water (0.018 kg mol^{-1}) and m_i the molality of solute i (moles per kg solvent).

However, foods, including honey are all but ideal solutions. Consequently, at the same water mole fraction X_w, water activity a_w can be quite different, depending on which solutes are present. Nonideal behaviour is accounted for by transforming Eq. 6 into Eq. 8:

$$a_w = \frac{p}{p_0} = \gamma_w X_w \tag{8}$$

The water activity coefficient γ_w accounts for nonideality (activity coefficients depend on the scale, so this one is for the mole fraction scale). Since theoretical relations are hard to establish, many empirical relations have been developed. Fortunately, water activity can be measured quite accurately from colligative properties such as boiling point elevation, osmotic pressure, freezing point depression. Subbiah et al. (2020) reviewed and analyzed the water activity of sugar solutions and model honeys. They used the concept of hydration numbers as the average number of water molecules that are bound to each solute molecule so that they do not contribute to water activity. Based on the parameters given in Subbiah et al. (2020) (their Fig. 2), the calculated relation between water activity and moisture content for glucose solutions is reproduced in Fig. 2, where the practical range of honey moisture contents is indicated by the dashed red lines. This analysis shows that, over this range, the relation is approximately linear, as reported in literature (e.g., Cavia et al., 2004; Chirife et al., 2006; Costa et al., 2013). Of course, honey is much more complicated than a simple glucose solution. While Fig. 2 is not a blue print for the relation in honey, it serves to show the general trend to be expected for the relation between water activity and moisture content and the range where a linear approximation might hold.

Crystallization can easily occur in honey as it is a highly concentrated solution; if that happens, water activity increases because of the release of water, resulting in a higher activity of water. However, supersaturation can easily occur also, so it is hard to predict what will happen. For the remainder of this paper, it will be assumed that the relation between water activity and moisture content in honey is approximately linear, as

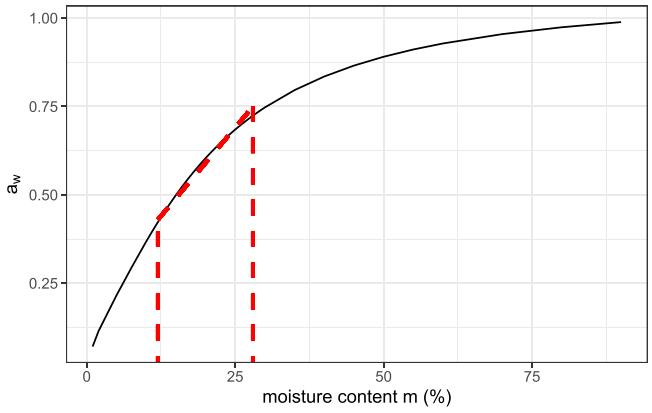

Fig. 2. Calculated relation between water activity a_w and moisture content m for aqueous glucose solutions according to the analysis of Subbiah et al. (2020). The dotted red lines indicate the region where a linear approximation over the practical moisture range for honey might hold (12% < m < 28%).

is done in the used literature sources.

2.4. Objectives

The first objective of this paper is to investigate and establish the relation between water activity and moisture content of honeys from different geographical origin. The hypothesis (based on existing literature) is that this relation can be described by a linear model, $y = a + bx$, where the slope b is the same for all honeys whereas the intercept a varies depending on the region of the honey. A Bayesian multilevel approach will be applied to investigate this hypothesis. The second objective is to investigate how a Bayesian multilevel approach can be used to characterize variability in honeys (and foods in general). The third objective is to investigate the type of predictions that can be made based on established relations between variables (water activity and moisture content in the particular case of honeys).

3. Material and methods

3.1. Material

Data were obtained from literature, either directly read from Tables or extracted from graphs using the freely available software WebPlotDigitizer. The data were coded according to their origin as shown in Table 1. All in all, 29 different suitable data sets were found for regression analysis, with a total of 1144 data points. A few data sets found in literature were not used because of exceptionally high moisture content and water activity values; as shown in Fig. 2, a linear relation is then no longer realistic. This concerned data from Lavinas et al. (2023) from the Atlantic Forest and Caatinga regions in Brazil; however, their data about the Cerrado region in Brazil with 'normal' ranges were included. Another excluded data set with unusual high moisture and water content was from Mokaya et al. (2022) for Kenyan samples, and yet another one from Ávila et al. (2019) for Brazilian honey. The unusual high values (up to 40% moisture content and a_w values up to 0.9) were also noted by the authors; interestingly, the data sets with these extreme values were all about stingless bee honeys. Another data set not used in regression analysis was that from Beckh et al. (2004) because those data were not from one specific origin but mixtures from all over the world. However, this data set was used in relation to validation so that thirty data sets were used in total.

Table 1
Overview of the origin of the honey data sets (random order).

Origin	Code	Reference
Slovenian (flower honey)	1	Abramovič et al. (2008)
Slovenian (honeydew)	2	Abramovič et al. (2008)
German (flower)	3	Gleiter, Horn, and Isengard (2006)
German (honeydew)	4	Gleiter, Horn, and Isengard (2006)
Spanish (mixed honeys)	5	Cavia et al. (2004)
La Palma	6	Sanjuan et al. (1997)
Argentinian	7	Chirife, Zamora, and Motto (2006)
Turkish (flower honey)	8	Serin, Turhan, and Turhan (2018)
Indian	9	Saxena, Gautam, and Sharma (2010)
Brazilian	10	Silva et al. (2013)
Tunesian	11	Boussaid et al. (2018)
Mexican	12	Viuda-Martos et al. (2010)
Colombian	13	Giraldo, Acosta, and Gallego (2013)
Mexican	14	Mondragón-Cortez et al. (2013)
Spanish	15	Sanz et al. (1995)
Brazilian	16	Da Silva et al. (2016)
Greek	17	Lazaridou et al. (2004)
Turkish	18	Bayram et al. (2021)
Moroccan	19	Bouhlali et al. (2019)
Tenerife	20	Bentabol Manzanares et al. (2011)
Slovakian	21	Kacaniova et al. (2012)
Polish	22	Kacaniova et al. (2012)
Argentinian	23	Acquarone, Buera, and Elizalde (2007)
Czech	24	Vorlová et al. (2005)
Turkish	25	Kayacier and Karaman (2008)
Cerrado region Brazil	26	Lavinas et al. (2023)
Moroccan	27	Bouddine et al. (2022)
Tenerife	28	Bentabol Manzanares et al. (2014)
Brazilian	29	Costa et al. (2013)
Worldwide	30	Beckh, Wessel, and Lullmann (2004)

3.2. Methods

R version 4.2.2 was used as software (R Core Team, 2020) using RStudio version 2022.12.0 (RStudio Team, 2020) and Quarto for literate programming (https://www.quarto.org). Data handling was done in the tidyverse ecosystem (https://www.tidyverse.org). For Bayesian regression the R package brms version 2.17.0 (Bürkner 2017, 2018) was used; this package uses the probabilistic software program Stan (Gelman et al., 2015, Carpenter et al., 2017) in the background. Graphs were produced using the R package ggplot (Wickham, 2016). For an overview of the R packages used, see the Supplement.

Bayesian regression is done via MCMC sampling in Stan and it always needs to be checked whether convergence did happen. There are several ways to do that (Kruschke, 2021). For all the results given in this paper it was checked that convergence was in order but the diagnostic results are not reported here to save space. Some diagnostic results are given in the Supplement.

Other necessary actions in the Bayesian framework are prior and posterior predictive checks. A prior predictive check implies that the model is tested based on the proposed priors without invoking the data yet. Because Bayesian models are generative, such predictions can be made. It should be checked that the prior allows a wide range of predictions but should prevent impossible values. Comparison of the proposed prior with the resulting posterior is also useful. A posterior predictive check implies that predictions are made from the posterior (i.e., based on model and data) and the check is whether or not these predictions overlap with the actual data. This can be done in various ways. Some examples are given in the Supplement.

The R code as well as the data sets used in this paper are freely available on the author's GitHub page https://github.com/tinyvanboekel/honey.

4. Results

4.1. Exploratory data analysis (EDA)

An overview of all the available data used for regression is given as a scatterplot in Fig. 3 (Supplement Fig. S1 shows the same data plotted per origin). Fig. 3 shows that: i) there is quite some variability between the various origins, ii) the relation between water activity a_w and moisture content m seems more or less linear, as anticipated. These two observations make it appropriate to apply i) multilevel modeling and ii) linear regression to find out how a_w and m are related.

4.2. A null model

The most basic model that can be applied to the data is a so-called null model, or an intercept-only model, or an unconditional model (unconditional because there is no conditioning on a predictor variable); such a null model results effectively in estimates of the mean and standard deviation of the sampled population. For the present study, a null model can be achieved in three ways: i) with all data pooled, ii) separately for every origin cluster (no-pooling) and iii) by partial pooling (multilevel modeling). Since Bayesian regression is applied, likelihood and priors must be proposed for the data and parameters, respectively, as in Eq. 9:

$$\begin{aligned} a_w &\sim \mathcal{N}(\mu, \sigma) \\ \mu &\sim \mathcal{N}(0.55, 0.1) \\ \sigma &\sim \text{exponential}(1) \end{aligned} \tag{9}$$

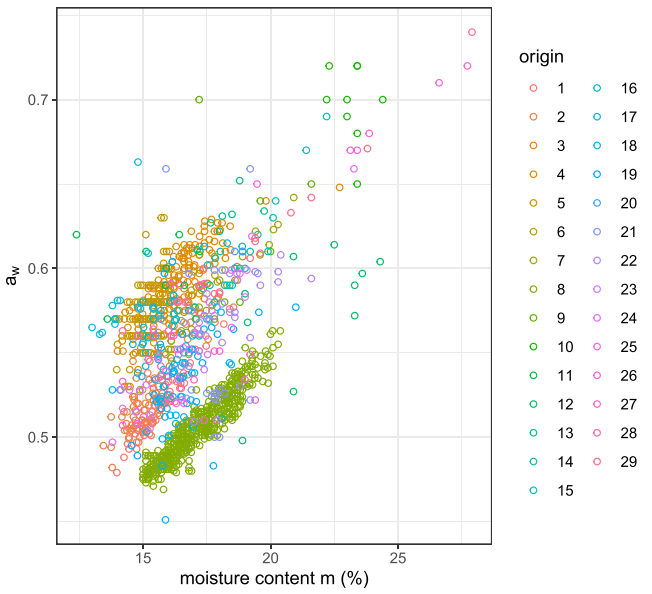

Fig. 3. Scatterplot of the 29 honey data sets used for regression (water activity a_w versus moisture content m) coloured per origin. Data sources are reported in Table 1.

The proposed likelihood for the data a_w is thus that they are normally distributed (symbol $\mathcal{N}$), while the suggested prior for the mean μ is also supposed to be normally distributed with a value of 0.55 (it is known from literature that the mean is approximately that value, i.e., this is prior knowledge) and a standard deviation of 0.1 (expressing the uncertainty about the actual value of the mean). Standard deviation σ is proposed to be exponentially distributed since it cannot be negative (actually, also the mean cannot be negative but with the parameter values proposed, $\mathcal{N}(0.55, 0.1)$, that will not be the case). Note that the standard deviation σ is used rather than the variance σ^2 because the Stan software works with standard deviations.

4.2.1. Complete pooling with the null model

Bayesian regression with completely pooled data resulted in the estimate a_w with mean value 0.547 (standard error 0.001, expressing the uncertainty in the estimate) while σ was estimated as 0.05 (standard error 0.001). The complete parameter posterior distributions can be found in the Supplement (Fig. S2), along with some diagnostic checks (Figs. S2-S6). The posteriors will be compared later on with other regression results. Supplement Fig. S5 provides a comparison of the proposed prior and the resulting posterior, showing that the posterior is much narrower than the prior. This indicates that the posterior is dominated by the data and that the prior acts as weakly regularizing (McElreath, 2020). Supplement Fig. S6 shows that posterior predictions match well with the observed data.

4.2.2. No-pooling with the null model

Analyzing every data set separately per origin yields 29 different estimates, using the same priors and likelihood as in Eq. 9. Considerable variation can be seen in the means indicated in Fig. 4 (closed black markers); this variation is of course also obvious from Fig. 3 but is now quantified. While the problem with complete pooling is underfitting (not all information in the data is used), no-pooling results in overfitting (too much trust in each data set, the result of one regression is not connected in any way to the next or previous regression). This is where partial pooling comes to the rescue as an alternative.

4.2.3. Partial pooling with the null model

Partial pooling via multilevel modeling acknowledges that the data do have something in common, while allowing also differences in the data. It is now assumed that the mean μ_j for each origin j varies around the global mean. This requires an extra parameter, namely the standard deviation σ_b that takes variation around the global mean (level 2) into account (i.e., the between-group variability): see Eq. 10. The assumption made in Eq. 10 is that the μ_j are normally distributed around the global mean μ. Parameter b_j (can be positive or negative) is the offset for each cluster from the global mean μ. Note that the overall σ is partitioned over two standard deviations σ_b (level 2) and σ_r (level 1).

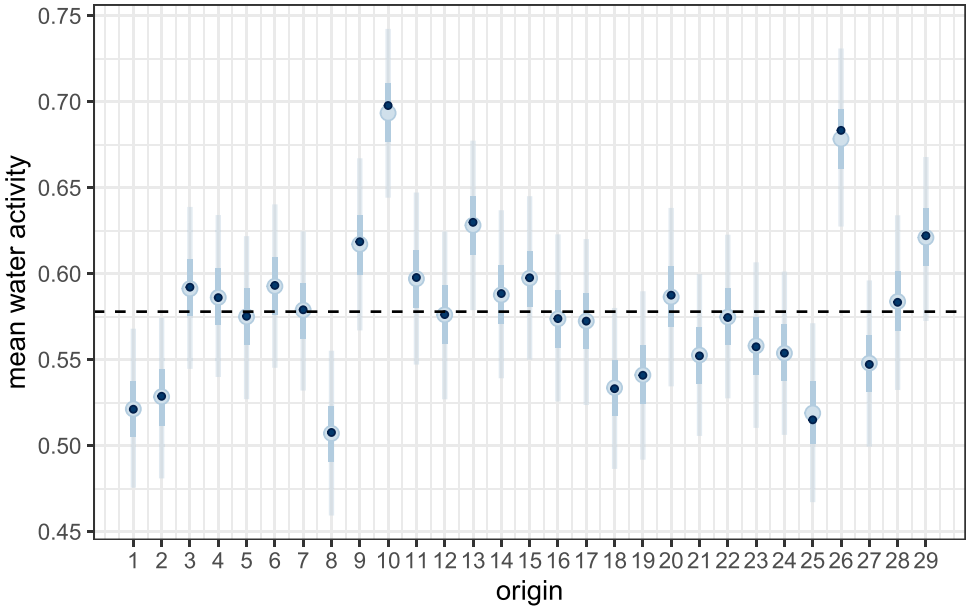

Fig. 4. Comparison of mean water activity estimates resulting from no-pooling (closed black markers) and partial pooling (open blue markers with 50 (thick line) and 95% credible interval (thin line)). The dotted horizontal line represents the overall population mean $a_w = 0.58$ resulting from partial pooling.

$$\begin{aligned} a_w &\sim \mathcal{N}(\mu_j, \sigma_r) \\ \mu_j &= \mu + b_j \\ b_j &\sim \mathcal{N}(0, \sigma_b) \\ \mu &\sim \mathcal{N}(0.55, 0.1) \\ \sigma_b &\sim \text{exponential}(1) \\ \sigma_r &\sim \text{exponential}(1) \end{aligned} \tag{10}$$

Posterior predictive checks can be found in the Supplement (Fig. S6) and were found to be OK. If parameter estimates differ from the overall mean, a phenomenon called shrinkage may be observed. It implies that parameter estimates shrink from their individual values towards the overall mean due to partial pooling. This may particularly happen for data sets with few data points. Fig. 4 compares parameter estimates resulting from partial pooling and no-pooling. Several data sets show such shrinkage effect, most clearly origins 9, 10, 13, 20, 25, 26, 29. Some data sets do not show visible shrinkage, meaning that there is no notable difference between the means obtained via individual regression and via multilevel modeling.

A graphic illustration of the null-model results can be found in Fig. 5, where the posterior distributions are compared resulting from complete pooling and partial pooling regression for the two parameters of the null model, the mean and the standard deviation. In Fig. 5A, two effects of partial pooling are visible: i) the estimate of the mean has shifted, and ii) the width (i.e., the uncertainty) of the estimate is much larger for partial pooling. While this latter effect seems to have worsen the situation, it is actually showing that the result of complete pooling gives a false impression of the uncertainty of the estimate. This is so because the effect of clustering is ignored, leading to underfitting: it is assumed that there is no variation between origins but this is clearly wrong. In Fig. 5B, the effects of partial pooling on the estimates of the population standard deviations are shown. As mentioned before, the total variance is now partitioned over the clusters and the remaining residual variance within the clusters, as a result of which the remaining variance of level 1 is much reduced while the variance due to clustering is seen to be quite high and moreover much wider (which indicates the uncertainty in this estimate). In passing, note that this shows the power of the Bayesian approach in visualizing parameter behaviour via posterior distributions, it gives much more insight than point estimates.

A summary of the posterior distributions resulting from partial pooling can be found in Table 2. σ_b is a quantitative measure for how the individual means bounce around a central value for each origin data set (the actual numerical offsets from the population mean (parameters b_j) are reported per origin in the Supplement, Table S1). When compared to complete pooling, the estimate for the intercept (i.e., the mean) has shifted from 0.55 to 0.58 while the standard deviation $\sigma = 0.05$ decreased because of partitioning over two levels. From σ_b and σ_r another interesting parameter called *ICC* (intra-class correlation coefficient) can be calculated as the proportion of the between-cluster variance and the total variance (see Eq. 11):

$$ICC = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_r^2} \tag{11}$$

$ICC = 0$ implies no variation between clusters, in other words,

Table 2
Results of Bayesian regression of the honey data using partial pooling with the null model. SE = standard error, lower and upper bound indicate 95% credible intervals.

parameter	mean	SE	lower bound	upper bound
μ	0.578	0.008	0.562	0.594
σ_b	0.045	0.007	0.034	0.061
σ_r	0.024	0.001	0.023	0.025

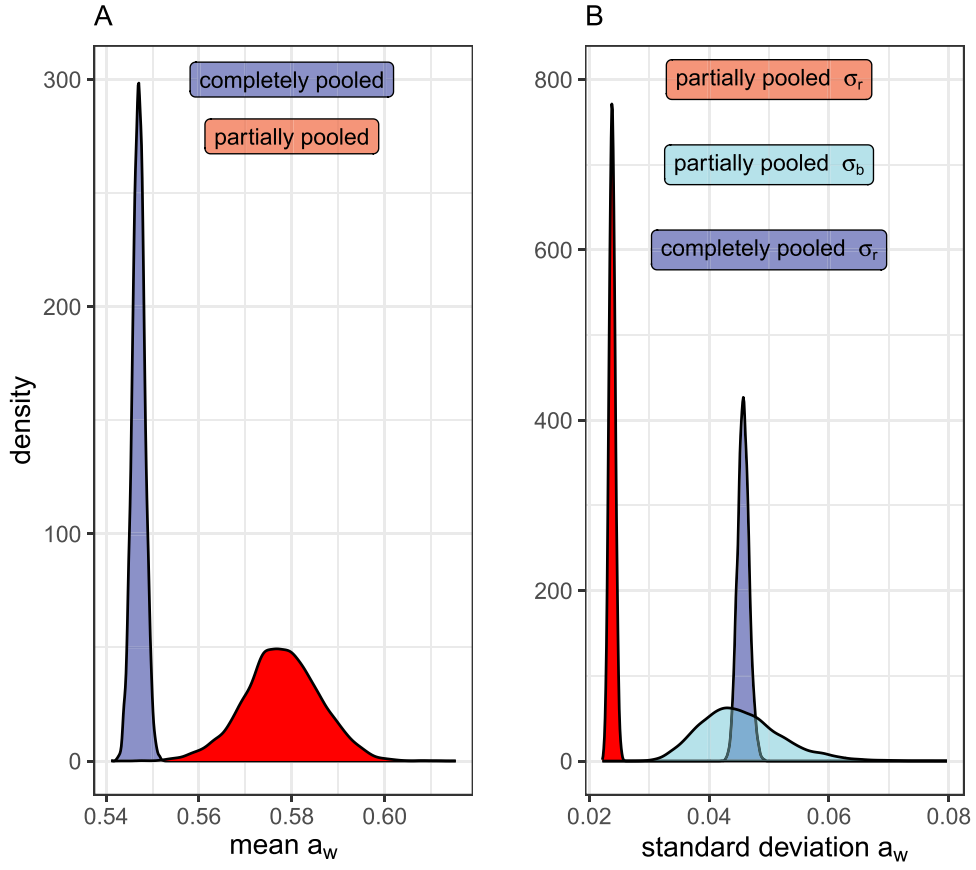

Fig. 5. Comparison of posterior parameter distributions (A: means, B: standard deviations) resulting from pooled and partially pooled regression with the null model. The width of the distributions indicates the uncertainties of the estimates.

measurement results do not depend on a specific cluster membership. $ICC = 1$ indicates that all variance is explained by clustering the data, measurement results completely depend on the cluster they belong to. $ICCs = 0.01, 0.05, 0.2$ are considered as small, intermediate and large indicators, respectively, of cluster dependence (Sommet and Morselli, 2021). The *ICC* for this honey case is 0.78, meaning that 78% of the variance is explained by clustering the data in origins and therefore it can be concluded that mean water activity strongly depends on the origin of the honey. Multilevel modeling is therefore very appropriate.

Of course, the results so far only concerned a null model and the next step is to add a predictor, namely water content.

4.3. Regression with moisture content as predictor

As is clear from literature and also obvious from Fig. 3, moisture content of honey can act as a linear predictor of water activity over the practical ranges considered for honey. However, when doing linear regression with the raw data, the intercept will be estimated at $m = 0$ and this estimate should be 0 from a physical point of view, because by definition $a_w = 0$ at $m = 0$. Because the curvature in the relation displayed in Fig. 2 is ignored by assuming a linear relation, the intercept will not be zero if linear regression is applied. So, such an intercept value has no physical meaning. Therefore, it is better to center the predictor variable m. The additional benefit is that this will virtually eliminate parameter correlation between slope and intercept (McElreath, 2020), which is beneficial for parameter estimation. Centering is simply achieved by subtracting the mean of the predictor variable from the predictor variable itself.

4.3.1. Complete pooling with predictor variable

The first attempt is, once again, to ignore the clusters in the data and to pool all data. So, the model proposed is as in Eq. 12 where the centered moisture content is indicated as m_c:

$$a_w = \beta_0 + \beta_1 \cdot m_c \tag{12}$$

Again, a likelihood function for the data and priors for the parameters need to be given, as suggested in Eq. 13:

$$\begin{aligned} a_w &\sim \mathcal{N}(\mu_i, \sigma_r) \\ \mu_i &= \beta_0 + \beta_1 \cdot m_{c,i} \\ \beta_0 &\sim \mathcal{N}(0.58, 0.1) \\ \beta_1 &\sim \mathcal{N}(0.05, 0.01) \\ \sigma_r &\sim \text{exponential}(1) \end{aligned} \tag{13}$$

Note the subtle difference in parameter μ_i, it is no longer μ referring to the overall mean of the whole population as in Eq. 9. Instead, μ_i refers to the expected value at each data point i with standard deviation σ_r referring to the spread around this mean value (σ_r is supposed to be the same for each data point). Furthermore, prior normal distributions are proposed for parameters β_0, β_1 and, as before, a prior exponential distribution for σ_r. Since the predictor variable is centered, the intercept will be around the mean (estimated to be 0.58 from the null model, with standard deviation 0.1), while the slope parameter is given an expected value of 0.05 with a standard deviation of 0.01. With these prior settings, a prior predictive check showed that a wide range of possible values is obtained without producing impossible values (Supplement Fig. S7).

Supplement Fig. S8 compares posterior distributions, pair plots and correlation coefficients for regression with non-centered (Fig. S8A) and centered predictor variable (Fig. S8B). It shows clearly that centering indeed removes strong parameter correlation between slope and intercept. Moreover, the intercept does have a physical interpretation upon centering, namely the mean a_w at the mean moisture content. Therefore, for the remainder of the article, centered moisture content m_c is used as predictor.

The resulting regression line is displayed in Fig. 6A. Obviously, the regression line cannot account for all data because of the large scatter due to honey origin. And there is another effect that disturbs the picture, perhaps less visible in Fig. 6A but becoming very clear when only part of the data (origin 1–8) is used in the regression: see Fig. 6B. This regression line does not seem to make sense at all. This is a consequence of a statistical phenomenon called Simpson's paradox and is the result of neglecting the fact that the observations are not independent but clustered. As shown, this can be very misleading; in the case of Fig. 6 B, the many data from origin 8 draw the regression line downwards, which completely disturbs the picture. While the many more data in Fig. 6A show less of this Simpson paradox, it still can be a lurking phenomenon, as shown below. A numerical summary of the posterior is shown in

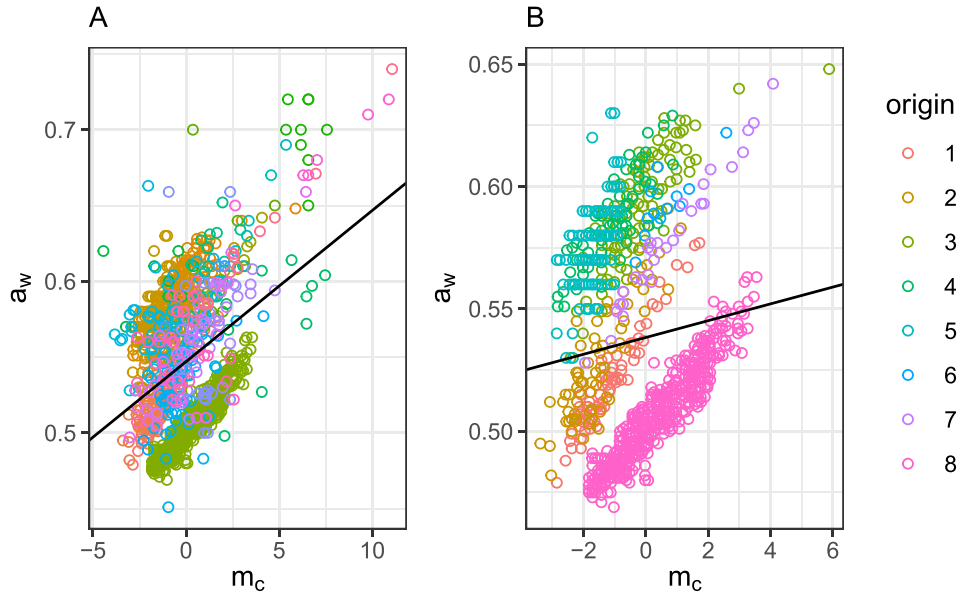

Fig. 6. Regression plot of all, completely pooled honey data (A) and of a subset (B, origin 1–8) to show the effect of Simpson's paradox using centered moisture content m_c as predictor.

Table 3
Numerical summary of Bayesian regression of the completely pooled honey data with centered predictor. SE = standard error, lower and upper bound indicate 95% credible intervals.

	Mean	SE	Lower bound	Upper bound
intercept β_0	0.547	0.001	0.54	0.55
slope β_1	0.010	0.001	0.01	0.01
σ_r	0.042	0.001	0.04	0.04

Table 3.

4.3.2. *No pooling with predictor variable*

No-pooling regression was done with the same priors and likelihood as for complete pooling (Eq. 13). While complete pooling does not acknowledge the fact that the data are clustered, no-pooling ignores the fact that the data have something in common, as already discussed above for the null model, leading to 29 different slopes and intercepts, displayed as dots-and-whisker plots in Supplement Fig. S9. The resulting

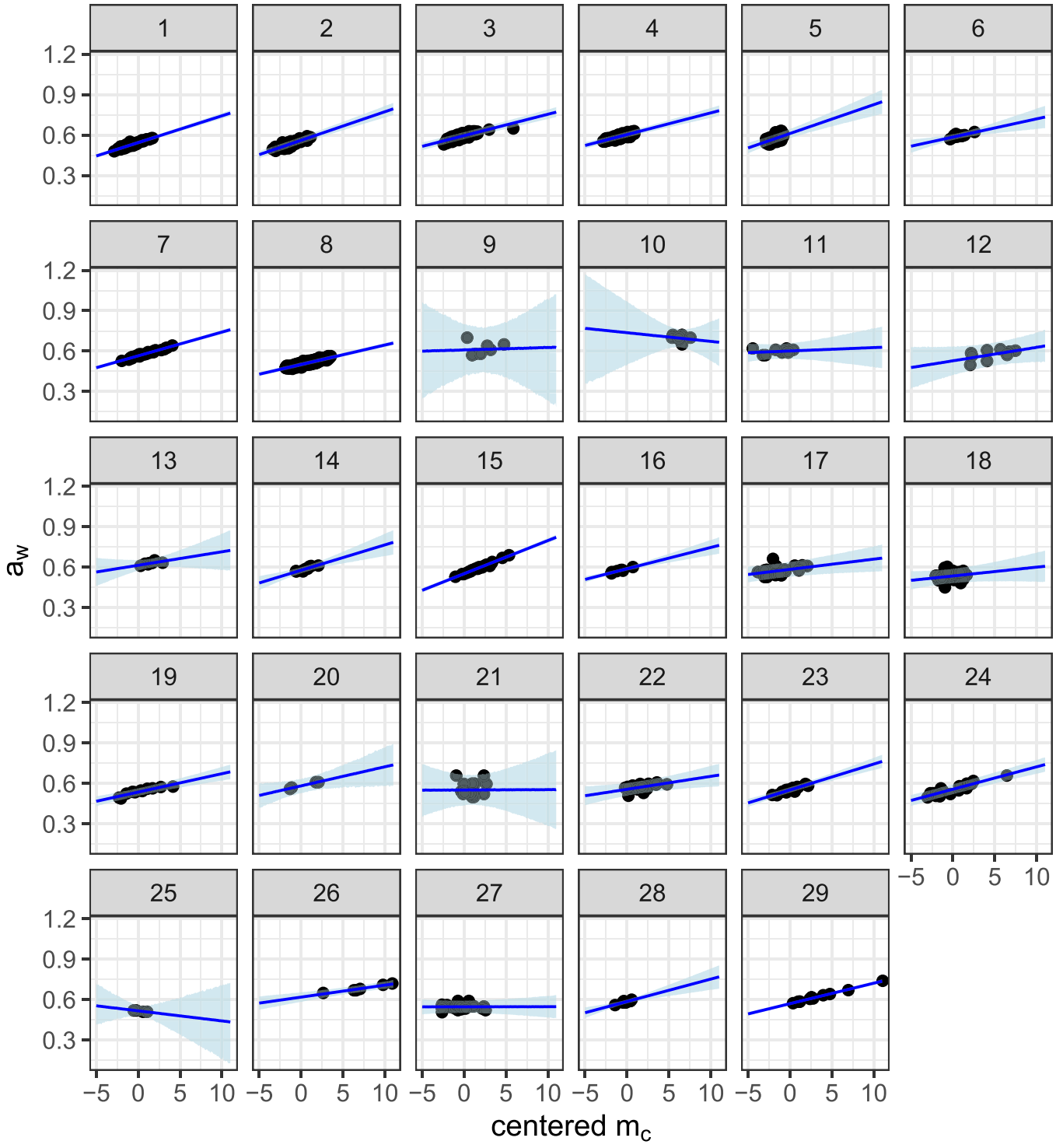

Fig. 7. Regression lines resulting from individual regressions (no pooling) of the honey data according to origin using centered moisture content m_c as predictor. The lightblue ribbons represent the 95% prediction intervals.

fits are shown in Fig. 7. While the individual fits are not bad, there are remarkable differences, note also the sometimes very wide prediction intervals. Regression results from some origins deviate quite strongly, notably origin 9, 10, 11, 21, 25 (the slopes for origins 10 and 25 are even negative). Most importantly, it is not possible to generalize using no-pooling results beyond the cases that were sampled, and for individual cases the predictive capacity is wildly different for each individual case as shown in Fig. 7.

4.3.3. *Partial pooling with predictor variable*

Since it is clear from literature as well as the results discussed above that variation is especially in the intercepts and hardly in the slopes, multilevel regression is attempted with varying intercepts only. This requires different priors and likelihood: see Eq. 14. b_{0j} is the offset for each origin j from the global intercept β_0 while β_1 is the global slope shared by all origins. σ_r describes the within-origin variation and σ_b the between-origin variation.

$$\begin{aligned} a_w &\sim \mathcal{N}(\mu_{ij}, \sigma_r) \\ \mu_{ij} &= (\beta_0 + b_{0j}) + \beta_1 \cdot m_{c,ij} \\ b_{0j} &\sim \mathcal{N}(0, \sigma_b) \\ \beta_0 &\sim \mathcal{N}(0.55, 0.1) \\ \beta_1 &\sim \mathcal{N}(0.05, 0.01) \\ \sigma_b &\sim \text{exponential}(1) \\ \sigma_r &\sim \text{exponential}(1) \end{aligned} \tag{14}$$

Posterior predictive checks were done and found to be OK: see Supplement Fig. S10, the posterior predictions match well with the actual data. As with the null model (Fig. 5), comparison of complete pooling and partial pooling can be done for the model with predictor via posterior parameter distributions: see Fig. 8. Partial pooling leads to larger, more realistic uncertainty for the overall intercept but to less uncertainty for the overall slope because the regressions have "informed each other". Also, it leads to substantially different estimate values.

A kind of posterior prediction check is to see in how far regression lines are able to match with the data upon which the model is based. McElreath (2020) calls this 'retrodiction', as opposed to prediction, because it shows in how far the model based on data match in retrospect. The regression line resulting from partial pooling with varying intercept is shown in Fig. 9 next to the one from complete pooling. The difference is not to be missed: partial pooling takes clustering into account while complete pooling does not and is subject to the effect of Simpson's paradox, as explained above. The regression line in Fig. 9 is plotted according to the overall population estimates for slope and intercept. The varying intercepts resulting from partial pooling are shown in Fig. 10. When compared to the ones in Fig. 4 for the null model, considerable variation is still seen between origins but less so than in Fig. 4; this is the result of shrinkage.

Fig. 11 shows fits for the separate data sets in three ways: i) fit at the population level obtained from partial pooling (same line for each data set), ii) fit at the group level obtained from partial pooling (different regression line for each group), iii) fit at the individual level obtained from individual regression (no pooling). Note that all data sets are well described by the same slope, implying that water activity varies with moisture content in the same way for all data sets, regardless of their origin. Obviously, fits *at the population level* are not always good at the individual level because it is a compromise between over- and

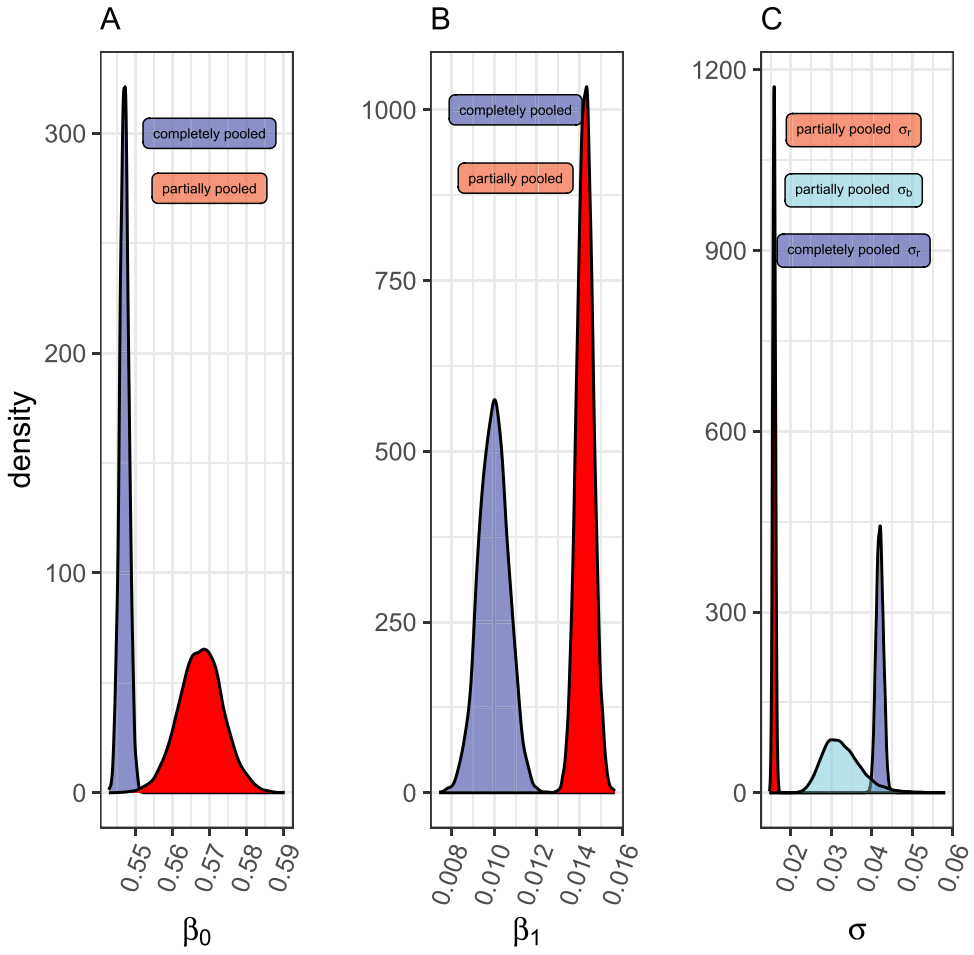

Fig. 8. Comparison of posterior parameter distributions resulting from regression with centered predictor for complete pooling and partial pooling. A: intercept, B: slope, C: standard deviations.

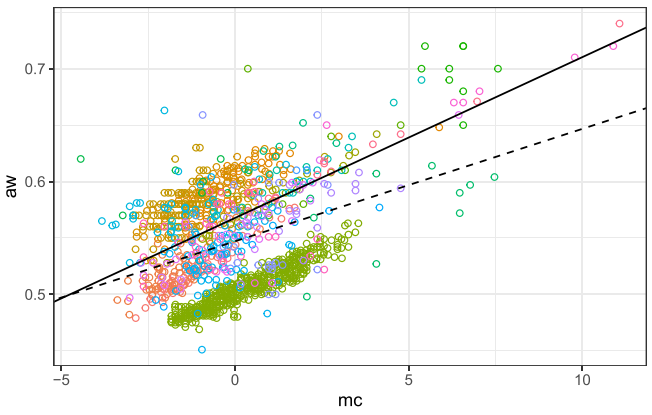

Fig. 9. Regression line resulting from partial pooling (solid line) and complete pooling (dashed line) for all 29 honey data sets.

underfitting. But this regression line will give the best result in predicting overall results. On the other hand, the fits *at the group level* are generally very good. Even though the *rate of change* in water activity with moisture content is the same for all honeys regardless of origin, the *actual water activity values* are strikingly different and do depend on geographical origin, which is accounted for by varying intercepts. This variation must be a consequence of the fact that the origin of honey has an effect on composition: it can be due to different type of sugars, salts and other components that have an effect on water activity.

As mentioned, the fits at the individual level from partial pooling are generally good, but especially for origins 5, 9, 10, 11, 15, 17, 18, 21, 25, 26, 27 one can see the power of multilevel modeling in Fig. 11. While the no-pooling results from individual regressions show a rather different regression line for these cases, the partial pooling results for these honeys follow the general trend of the other data sets. This is because the regressions are connected, they share information and "borrow strength from each other" (McElreath, 2020).

The numerical summary of the posterior distribution resulting from partial pooling is shown in Table 4. The actual offsets b_{0j} from the global parameter β_0 for each origin can be found in the Supplement Table S2. Compared to complete pooling summarized in Table 3, the population estimate for the intercept has shifted from 0.55 (complete pooling) to 0.57 (partial pooling), while parameter $\sigma_r = 0.042$ (complete pooling) has decreased to 0.016 because of partitioning of variance towards σ_b. In other words, most of the variance is attributed to between-cluster variance. This is also indicated by the *ICC* parameter, which now has two values, an adjusted $ICC = 0.79$ that only takes varying effects into account, and an unadjusted $ICC = 0.51$ that takes both varying and population effects into account. Both parameters still show convincingly a very strong effect of variance partitioning due to clustering of the data, so multilevel modeling is really appropriate.

5. Discussion: multilevel posterior predictions

As already mentioned above, fits to data on which regression is based are not predictions but rather retrodictions. Real prediction is to compare model outcomes with data that were not used to build a model. The question to be discussed now is how to make predictions from multilevel modeling. The answer is that it depends on the goal. An important benefit of multilevel modeling is that it prevents over- and underfitting (McElreath, 2020). If the goal is to make predictions at the overall population level ("the average honey"), then the population parameter estimates should be used. In statistical multilevel jargon, these are marginal effects: what is the effect of a variable (here: moisture content) across clusters on the response variable (here: water activity). If the goal is to make predictions at existing origin levels, then the group parameter estimates should be used. It is also possible to make predictions for new clusters that are not yet investigated. All the information to do such calculations is present in the joint parameter posterior distribution.

5.1. Global predictions and model honey

To predict the relation for an "average honey", the global estimates from partial pooling must be used. Two types of prediction can then be made: i) for the expected mean, and ii) for new not yet observed samples. To predict the range where the mean can be expected, the uncertainty in parameters β_0 and β_1 must be used. This is shown in Fig. 12A as the dark blue ribbon. To predict the range where new samples may be expected, also the sampling variability represented by σ_r must be taken into account, leading to a wider interval. This is shown in Fig. 12A as the light-blue ribbon. To validate these predictions, data from model honey

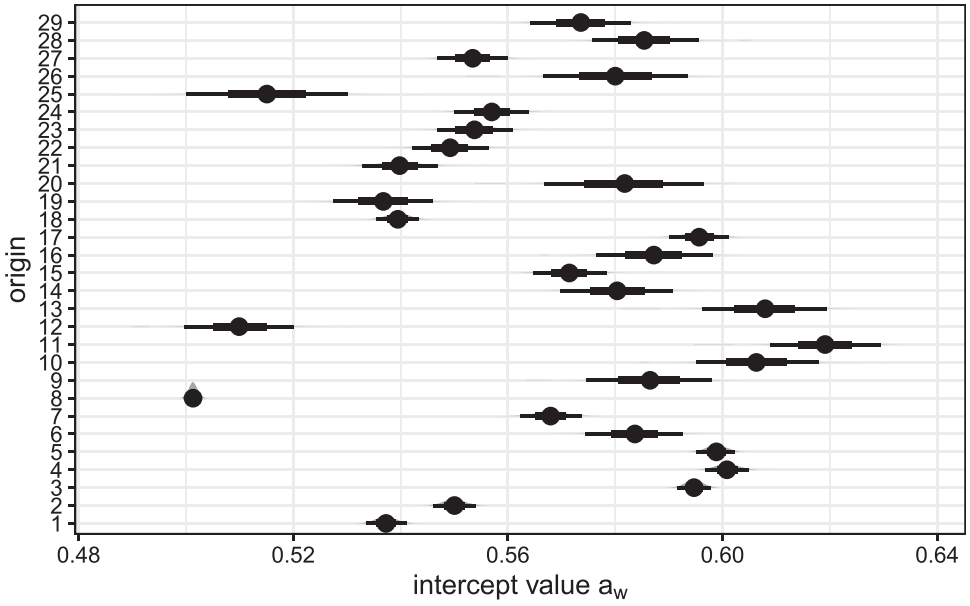

Fig. 10. Coefficient plot of the intercepts for each origin resulting from partial pooling with centered moisture content as predictor. The dots indicate the mean, the thick band the 50% and the small band the 95% credible interval.

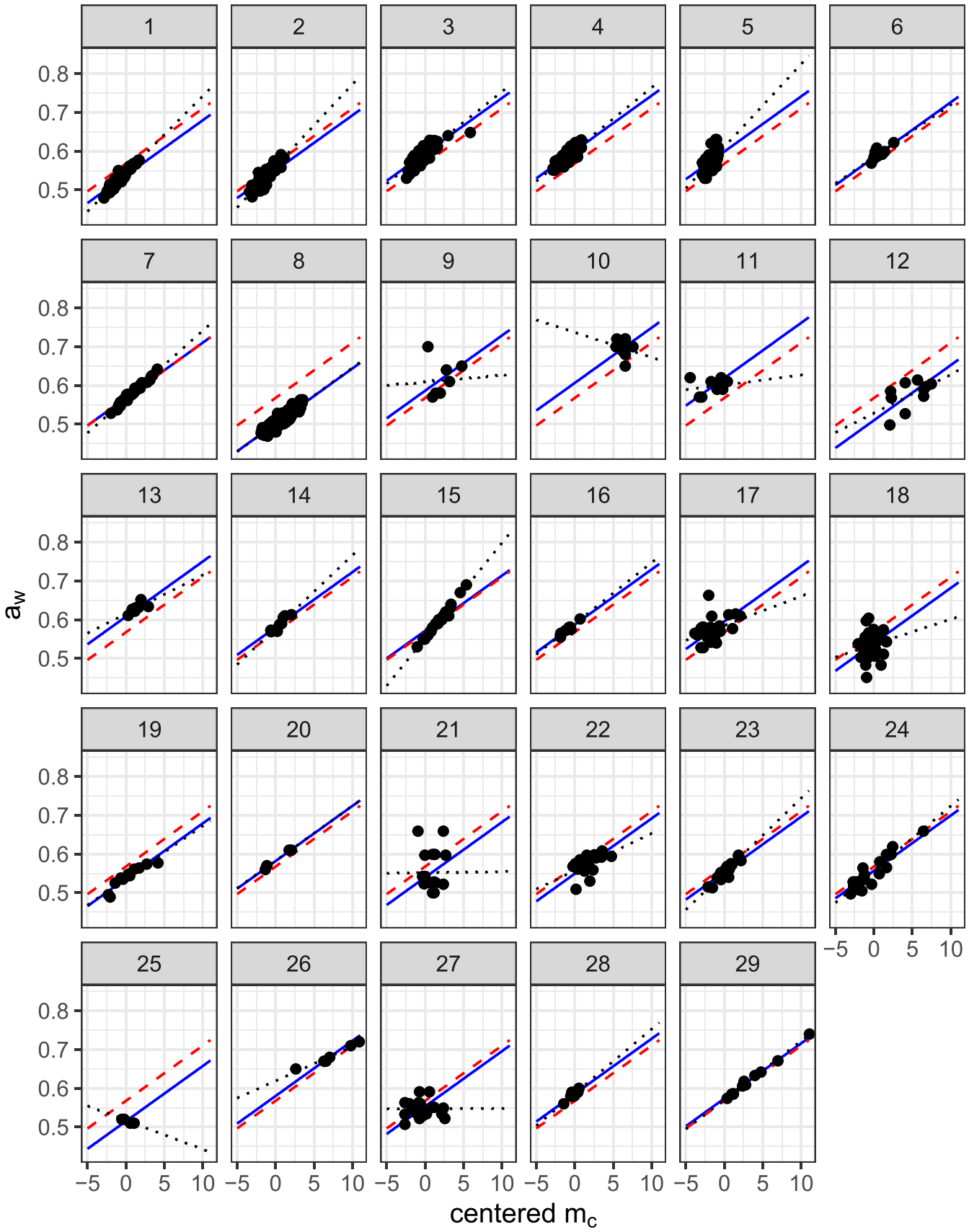

Fig. 11. Regression lines for the individual honey data sets. Solid blue line: group level (fit from partial pooling), dashed red lines: population level (fit from partial pooling, same for each data set), black dotted lines: individual regression (fit from no pooling).

Table 4
Results of Bayesian regression of water activity versus centered moisture content using partial pooling. SE = standard error, lower and upper bound indicate 95% credible intervals.

parameter	mean	SE	lower bound	upper bound
intercept β_0	0.568	0.006	0.556	0.580
slope β_1	0.014	0.000	0.014	0.015
σ_b	0.033	0.005	0.025	0.044
σ_r	0.016	0.000	0.015	0.017

will be used, as discussed next.

It has been stated repeatedly in literature (e.g., Zamora et al. (2006) that the relation between water activity and moisture content is mainly determined by sugar content and composition (mainly glucose and fructose and to a lesser extent by disaccharides as sucrose and maltose). Such a relation has been investigated experimentally by Ruegg and Blanc (1981) (using mixtures of glucose, fructose, maltose and sucrose), Zamora et al., 2006 (mixtures of glucose and fructose) and Subbiah et al., 2020 (same mixtures as Ruegg and Blanc, 1981). Fig. 12A compares the prediction from the partially pooled regression at the population level (with centered moisture content as predictor) with these model mixtures. The model honey data appear by and large to coincide with the regression line obtained from multilevel modeling (predicted at the population level, so ignoring cluster effects) over the moisture range valid for honeys; they are close to or within the 95% credible interval (the range where the mean can be expected with 95% probability) and completely within the 95% prediction interval (the range where all future values may be expected with 95% probability). This is real prediction because the model honey data were not involved in building the model, and so the prediction is actually amazingly good. To compare the model honey data with real honey data, Fig. 12B compares them (with moisture content as predictor) and the model honey data indeed seem to represent "average honey".

Thus, Fig. 12A confirms that the *rate of change* of water activity with moisture content is largely determined by glucose and fructose. Clearly, this is true at the overall population level but the present analysis also makes clear that there are considerable differences in actual water activity values according to the origin of honey (as shown quantitatively in Fig. 10). Reasons for these differences must be due to minor components such as salts and saccharides other than glucose and fructose. According to Subbiah et al. (2020), the main effect of salt, for instance, is to lower the mole fraction of water rather than water activity and they concluded that for a food system with multiple sugars and electrolytes the data is best represented by mole fraction of water rather than by moisture content. Unfortunately, for the data sets considered here, data about full composition are lacking and so it is not possible to calculate mole fractions of water. These are hard to predict beforehand, of course, when it is not known what the composition is in terms of minor components. However, the present multilevel analysis has shown that the slope of the linear water activity - moisture content relation can be considered generally valid, while it also makes it possible to quantify the deviations in actual water activity values in terms of varying intercepts. A word of caution, however, extrapolation beyond the practical range of moisture contents is dangerous because the linear approximation will no longer hold (a hint of this is also suggested by the experimental model honey data in Fig. 12B), while this may also be questionable for higher moisture contents, see also Fig. 2.

5.2. Prediction for honey from existing origins

If the interest is in predicting the a_w - m_c relation for honeys from an origin that was included in the model building, then those specific parameters can be used to predict the range where new data may be expected *for that specific origin*. In statistical multilevel jargon, these are conditional effects, the effect of a variable (here: moisture content) on the average outcome a_w for a typical sampled cluster. A 95% prediction band is shown in Fig. 13 as the red ribbon for a typical honey on average (origin 29 was used for illustration because its individual regression line coincides with the population regression line, see Fig. 11).

5.3. Prediction for honey from unknown origins

A multilevel model can also be used to make predictions for new clusters that have not yet been sampled. In contrast to existing samples, no specific information is available for new origins, obviously, but nevertheless estimates can be made because there is general information about the global population of honey samples due to multilevel modeling and the variation around the global mean. Simulations can be made from the posterior, which requires three sources of variability:

- within-group sampling variability
- between-group sampling variability
- variability in the global parameters

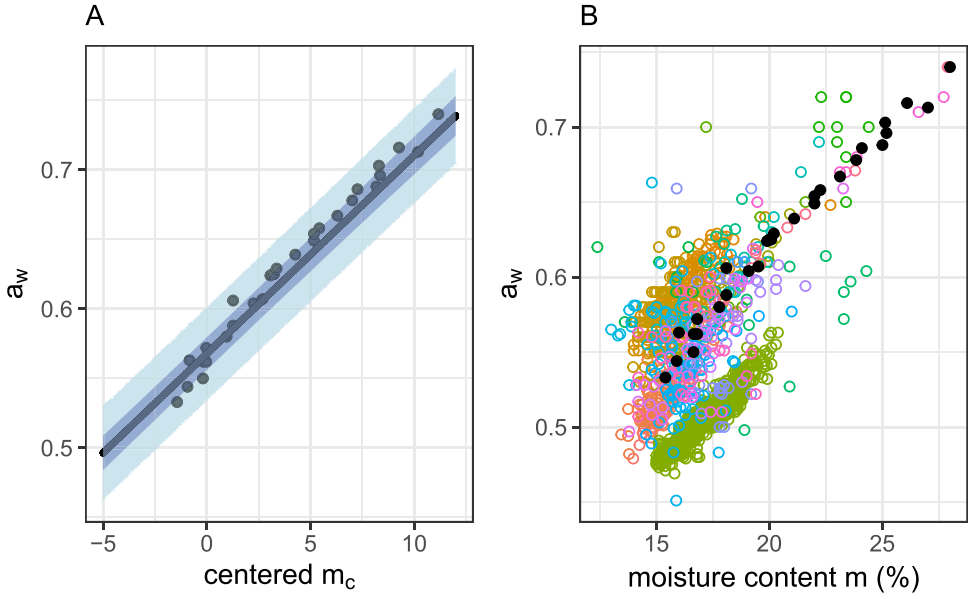

Fig. 12. A: Prediction of "average honey" using global regression parameters from partial pooling with centered predictor. Black line: regression line, dark-blue ribbon: 95% credible interval, light-blue ribbon: 95% prediction interval. Model honey data compiled from Ruegg and Blanc (1981), Zamora et al. (2006) and Subbiah et al. (2020) (closed black symbols) are shown for comparison. B: Comparison of water activity of honey data (coloured open markers) with those of model honey data (closed black markers) as a function of moisture content.

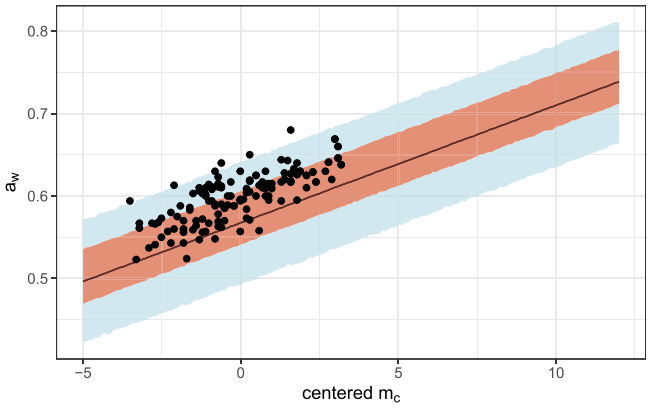

Fig. 13. 95% prediction intervals around the regression line for new samples from existing origin 29 (red ribbon) and for samples coming from a new, unknown origin (lightblue ribbon). The data points are for honeys from all over the world collected by Beckh et al. (2004); the data points are not connected to the model.

Fig. 13 shows the 95% prediction interval as a lightblue ribbon for a new, not yet sampled origin. Obviously, the prediction interval for unknown origins is (much) wider than for existing ones but nevertheless informative. The fact that such predictions can be made is a big advantage of multilevel modeling. As mentioned above, a large data set is available from Beckh et al. (2004) with samples from all over the world. This data set was not used in the model building, so it is interesting to see in how far model predictions cover these observed data; the data set is therefore plotted in Fig. 13. Even though the regression line seems to underfit somewhat, the data appear to be almost completely contained within the 95% prediction band. Zamora et al. (2006) commented on the dispersion in the data set from Beckh et al. (2004): "we are inclined to believe that a lack of accurate measurements of water activity may have been a reason for the poor goodness of fit observed in some cases". Whatever the reason, with the current analysis it seems that the data from Beckh et al. (2004) still fall within the overall population trend.

5.4. *Research options for multilevel modeling*

The possibility of doing multilevel modeling depends obviously on how samples were collected. In the present study, data from specific geographic origins were available and so two-level modeling could be applied. Further differentiation could be obtained within a specific region and with different bee species but such data were not available to the best of author's knowledge. For further research it could be interesting to have a sampling strategy that accounts for clustering the data. In principle, three or four level modeling could easily be applied with the proposed modeling procedure.

While the case study was about honey, multilevel modeling should be equally beneficial for investigating other foods, as they are inherently variable in composition and characteristics. The technique allows to quantify this variability, which is of great practical significance. For the case of honey, a model with *varying intercepts* and a *fixed* slope was found to be applicable. However, multilevel modeling also allows to handle cases where both slopes AND intercepts are variable in linear models, or for varying parameters in nonlinear models (e.g., Van Boekel, 2021a; Van Boekel, 2021b; Van Boekel, 2022; Garre et al., 2022). In fact, according to McElreath (2020), multilevel regression should be the standard rather than the hitherto commonly applied single level regression.

6. Conclusion

Multilevel modeling has confirmed the linear relation between water activity and moisture content (over the practical range of moisture content of honey from 12% to 27% or so) (first objective). The slope of this linear relation can be considered constant across geographical origins while the intercepts (reflecting the actual water activity values) are dependent on the origin of honeys. The extent to which they vary has been quantified now. So, multilevel modeling allows to get insight in the broader population of honeys (second objective). It is also beneficial for statistical reasons because it takes interdependence of data into account. Consequently, more realistic impressions of variability have become available than the ones obtained from -hitherto- single level modeling. Pooling of data, as well as individual regressions according to origin can be quite misleading in terms of estimates as well as in uncertainty estimates. Multilevel modeling through partial pooling gives the best compromise between over- and underfitting. Thus, this modeling technique allows prediction at the overall population level as well as at the level of clusters according to origin (third objective).

The current paper has shown that there is much to gain by applying multilevel modeling because it gives much more insight in data and their variability and counteracts statistical artefacts. Moreover, the Bayesian approach gives more insight into parameter behaviour than the classical frequentist approach. It makes parameters visible via probability distributions, it quantifies uncertainty about hypotheses and parameters and is much more geared towards prediction than the frequentist approach. Thus, it is concluded that the combination of multilevel modeling and the Bayesian approach leads to better predictive capacity of models to describe food characteristics.

Supplemental material

The Supplement contains additional graphs and tables, regression diagnostics as well as a complete list of the R packages used. The R code used for analysis as well as the raw data can be found at the author's Github page (https://github.com/TinyvanBoekel/honey).

Declaration of Competing Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data are made available at my GitHub page as mentioned in the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105595.

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