

## Uncertainty analysis of hygrometer-obtained water activity measurements of saturated salt slurries and food materials

Xin Yu <sup>a,1</sup>, Arthur R. Schmidt <sup>b,2</sup>, Shelly J. Schmidt <sup>c,\*</sup>

<sup>a</sup> Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 399B Bevier Hall, 905 South Goodwin Ave., Urbana, IL 61820, United States

<sup>b</sup> Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, 2521 Hydrosystems Laboratory, 205 N. Mathews, Urbana, IL 61801, United States

<sup>c</sup> Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 367 Bevier Hall, 905 South Goodwin Ave., Urbana, IL 61820, United States

### ARTICLE INFO

#### Article history:

Received 19 June 2008

Received in revised form 4 November 2008

Accepted 1 December 2008

#### Keywords:

Water activity

Hygrometer

Dew point

AquaLab

Uncertainty analysis

### ABSTRACT

Water activity is a critical food stability parameter. Thus, assessment of the uncertainty associated with the instruments and measurement procedures used to obtain  $a_w$  data is critical and can be accomplished using uncertainty analysis. Uncertainty analysis was applied to  $a_w$  data obtained for two saturated salt slurries (potassium acetate and potassium chloride) and two food materials (corn flakes and grape jam) at 25 °C using a commercially available dew point hygrometer, the AquaLab 3TE. Sources of uncertainty investigated included sample and dew point temperatures, sample lot, cup volume, and sample preparation (“as is” or ground), and day to day changes. Uncertainty analysis revealed that the magnitude of the different sources of uncertainty depended on material type, but that the dew point hygrometer instrument uncertainty was always significantly smaller than each of the other sources of uncertainty investigated, except for jam cup volume, which was exceeding small. Uncertainty analysis also revealed that dew point hygrometer accuracy was better than the  $\pm 0.003 a_w$  value reported by the manufacturer. Measurement procedures were identified that could be implemented to reduce  $a_w$  measurement uncertainty for corn flake and jam food materials. The uncertainty analysis used herein can also be applied to investigate other food materials and/or instruments.

© 2008 Elsevier Ltd. All rights reserved.

### 1. Introduction

Water activity ( $a_w$ ) is defined as the ratio between the vapour pressure of water in equilibrium with a sample and the vapour pressure of pure water at the same temperature and atmospheric pressure. Since its early use by Christian and Scott (1953), Scott (1953, 1957), and despite its limitations (Franks, 1982, 1991; Slade & Levine, 1991, and discussed in Schmidt (2004)),  $a_w$  has been successfully correlated with the prediction of food stability and safety and is, thus, one of the most important parameters with respect to microbial growth, rates of deteriorative reactions, and physical properties. Therefore, assessment of the uncertainty, including accuracy and precision, of instruments and measurement procedures used to obtain  $a_w$  data is critical. Water activity instrumentation accuracy can be determined by comparing instrument  $a_w$  readings to literature  $a_w$  values for saturated salt slurries and  $a_w$  instrumentation precision can be calculated as the standard deviation across multiple  $a_w$  sample readings. Assessment of the magni-

tude of the sources of  $a_w$  measurement uncertainty, however, is more complicated and is accomplished through application of uncertainty analysis.

As stated in the International Organisation for Standardisation (ISO) Guide to the Expression of Uncertainty in Measurement (ISO, 1995), the uncertainty of a measurement is defined as a “parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand”. This “parameter” can be defined in a number of ways, but is often defined, as it is in this study, as the standard deviation. In practice, there are many possible sources of uncertainty in a measurement, including, but not limited to, those identified by van Zoonen, Hoogerbrugge, Gort, van de Wiel, and van't Klooster (1999) – sampling protocol, matrix effects and interferences, instrument resolution or discrimination threshold, inaccuracy of measuring equipment, and values of constants and other parameters obtained from external sources.

The four basic steps used to determine measurement uncertainty, using the ISO approach as summarised by Maroto, Boque, and Ruis (1999) are identification, specification, quantification, and combination. The identification step requires the researcher to distinguish all of the uncertainty parameters within the analytical method that can influence the resultant measurement. The specification step requires the researcher to establish the mathematical

\* Corresponding author. Tel.: +1 217 333 6963.

E-mail addresses: [xinyu77@gmail.com](mailto:xinyu77@gmail.com) (X. Yu), [aschmidt@uiuc.edu](mailto:aschmidt@uiuc.edu) (A.R. Schmidt), [sjs@uiuc.edu](mailto:sjs@uiuc.edu) (S.J. Schmidt).

<sup>1</sup> Tel.: +1 217 244 2719.

<sup>2</sup> Tel.: +1 217 333 2978.

## Nomenclature

$a_w$	water activity	$u_v$ and $u_v^2$	$a_w$ cup volume uncertainty and variance
$T_d$	dew point temperature	$u_s$ and $u_s^2$	$a_w$ sample preparation uncertainty and variance
$T_s$	sample temperature	$u_c$ and $u_c^2$	$a_w$ overall combined standard uncertainty and variance
$u^2(T_d)$	dew point temperature variance		
$u^2(T_s)$	sample temperature variance		
$u_m$ and $u_m^2$	$a_w$ measurement uncertainty and variance	$u_e$ and $u_e^2$	$a_w$ combined variance and uncertainty excluding targeted source
$u_i$ and $u_i^2$	$a_w$ instrument uncertainty and variance	$\Omega$ or $\Omega$	coefficient of variation
$u_o$ and $u_o^2$	$a_w$ other sources uncertainty and variance		
$u_d$ and $u_d^2$	$a_w$ day to day uncertainty and variance		
$u_l$ and $u_l^2$	$a_w$ lot to lot uncertainty and variance		

model of the measurement process, which determines the analytical result. The quantification step requires the researcher to determine the variance associated with each parameter. The combination step requires the researcher to calculate the overall uncertainty by combining the uncertainties of each parameter. The combined standard uncertainty of the measurement result  $y$ , designated by  $u_c(y)$ , which represents the estimated standard deviation of  $y$ , is the positive square root of the estimated combined variance  $u_c^2(y)$ , which can be estimated using Eq. (1) (ISO, 1995):

$$u_c^2(y) = \sum_{i=1}^N [c_i u(x_i)]^2 \quad (1)$$

Eq. (1) is based on a first-order Taylor series approximation of  $y = f(x_1, x_2, \dots, x_N)$ , assuming the input estimates,  $x_i$ , are uncorrelated, and is termed the law of propagation of uncertainty (ISO, 1995).  $c_i$  is the sensitivity coefficient for input variable  $x_i$ , which is estimated as  $c_i = \partial y / \partial x_i$ , the partial derivative of  $y$  with respect to  $x_i$  and  $u(x_i)$  is the standard uncertainty associated with the measured variable  $x_i$ . Since  $u_c^2(y)$  is a function of several separate uncertainty estimates, it is referred to as the combined standard uncertainty method. If  $u_c^2(y)$  is obtained from experimental measurements and then used to back calculate an estimate of a separate uncertainty source, it is referred to as the inverse combination method. Both the combined and inverse combination uncertainty methods were employed in this study.

Through the application of uncertainty analysis to  $a_w$  measurements,  $a_w$  results can be viewed as a range of expected values within which the true  $a_w$  of the sample is expected to fall, with a specified level of confidence, instead of a single, fixed value. The size of this range, and hence the confidence in the  $a_w$  value, are influenced by sources of measurement uncertainty. Based on our extensive experience, as well as input from others, we have identified a number of possible sources of measurement uncertainty associated with hygrometer-obtained  $a_w$  values for each type of material studied here. We have divided these possible sources of uncertainty into three major categories: those associated with the instrument, those associated with the sample, and those associated with temporal or day to day changes. Investigation of the uncertainty associated with the nature of the sample is especially important in samples of biological origin, since they are often quite heterogeneous, both compositionally and spatially. By better understanding the sources of uncertainty associated with hygrometer-obtained  $a_w$  measurements, food researchers and food industry personnel can develop more robust measurement procedures and protocols, which in turn will allow for more informed product development, quality assurance (including safety), and product shelf-life decisions.

Hygrometers are often the instrument of choice for measuring  $a_w$  values of food materials in both food industry settings and re-

search labs, because they are easy to use and provide rapid results. There are two basic types of hygrometers – electric hygrometers, which are based on electrical properties (i.e., resistance and capacitance) sensors and chilled (or cooled) mirror dew point hygrometers, which are based on the measurement of sample and dew point temperatures (Fontana, 2007). The hygrometer selected for use in this study, the AquaLab 3TE system, utilises the chilled mirror dew point technology. Only a few studies were found that investigated the accuracy and precision of dew point hygrometers, individually (Richard & Labuza, 1990; Roa & Tapia de Daza, 1991) or in comparison to other  $a_w$  measurement technologies (Love, 2004; Voysey, 1993). No literature studies were found that applied uncertainty analysis to investigate the sources of uncertainty associated with dew point or electric hygrometer-obtained  $a_w$  values. It is worth noting that Richard and Labuza (1990) investigated some of the parameters affecting the performance of a dew point hygrometers, including instrument warm up time, sample cup fullness, and abrupt changes in  $a_w$  between samples, however, they did not use uncertainty analysis to analyze their data. Thus, the objectives of this research were to (1) apply uncertainty analysis to identify and quantify sources of uncertainty associated with dew point hygrometer-obtained  $a_w$  values, (2) utilise the information gleaned from uncertainty analysis to reduce  $a_w$  measurement uncertainty and optimise measurement procedures, and (3) determine the accuracy and precision of the dew point hygrometer using the data collected for uncertainty analysis of the saturated salt slurries. It is important to note that a single hygrometer was selected in this study to serve as an example instrument to demonstrate the exceedingly useful data analysis capabilities of uncertainty analysis, which can forthwith be applied to other food samples and/or instruments, including but not limited to, other hygrometers.

## 2. Materials and methods

### 2.1. Samples

Four materials – two saturated salt slurries and two foods – were used in this study. The two saturated salt slurries were saturated potassium acetate ( $\text{CH}_3\text{COOK}$ ) and saturated potassium chloride (KCl). Saturated salt slurries represent materials that exhibit minimal sample variability. Potassium acetate and potassium chloride salts were specifically selected because they have  $a_w$  values similar to those of the two food materials studied. The saturated salt slurries were prepared by adding an excess amount of salt (ACS Reagent Grade, Sigma–Aldrich, St. Louis, MO) to distilled, deionised water. The solution was heated to about 50 °C and stirred on a stirring hot plate for about 2 h, then cooled to 25 °C. The two food materials were Kellogg's corn flakes (Kellogg Company,

Battle Creek, MI) and Smucker's Concord grape jam (The J.M. Smucker Company, Orrville, Ohio). These food materials were selected because they represent two extremely different material types – a heterogenous, low-moisture (glassy), particulate material (corn flakes) and a homogenous, relatively high-moisture, continuous material (jam). Sample preparation for corn flakes was “as is” (~1–3 cm flake diameter) and ground (~1–2 mm flake diameter), with grinding being carried out via gentle breaking of the flakes using a mortar and pestle. Between the day to day  $a_w$  measurements, samples were capped with sample cup lids, tightly wrapped with parafilm, and then sealed in Ziploc<sup>®</sup> plastic bags to minimise moisture migration between the sample and the environment and then stored in a constant temperature chamber set at 25 °C.

## 2.2. Instrument

The AquaLab 3TE (serial number 0600696, model A)  $a_w$  metre manufactured by Decagon Device Inc. (Pullman, WA) was selected as the example hygrometer, since it is the main type of hygrometer used in the Schmidt lab. The chilled mirror dew point technology employed by the AquaLab determines the  $a_w$  of a sample by measuring the dew point temperature of the air ( $T_d$ ) in the chamber headspace and the temperature of the sample ( $T_s$ ) once vapour equilibration has been achieved. The dew point temperature is used to calculate the vapour pressure of the air in equilibrium with the sample, since, by definition, the saturated vapour pressure at the dew point temperature is equal to the vapour pressure at ambient temperature (Fontana, 2007; Water Activity News, 2006). The sample temperature is used to calculate the saturated vapour pressure at the same ambient temperature. Water activity is then determined by dividing the saturated vapour pressure at the dew point temperature by the saturated vapour pressure at the sample temperature. Detailed procedures for making  $a_w$  measurements using an AquaLab instrument (henceforth termed dew point hygrometer) are given below (AquaLab Operator's Manual, 2002).

A sample cup (made of HDPE; internal radius 1.94 cm, internal height 1.09 cm, and sample volume 13.0 cc) containing the sample is placed in the dew point hygrometer sample drawer. The sample drawer is subsequently closed and sealed and the water in the sample is allowed to equilibrate with the water vapour in the sample chamber headspace. It is critical for the water in the sample to control the vapour pressure of the chamber headspace, since the calculated  $a_w$  value is dependent on this equilibration being dominated by the water in the sample. At equilibrium, the vapour pressure of the air in the chamber should be equal to the vapour pressure of the sample. Next, to determine this equilibrium vapour pressure, the dew point and sample temperatures are measured.

The surface temperature of the sample is measured using an infrared thermometer and the dew point temperature is measured with a thermocouple attached to a mirror, which is monitored by a photoelectric cell (located in the chamber). The dew point hygrometer mirror temperature is precisely controlled by a thermoelectric (Peltier) cooler. Detection of the exact point at which condensation first appears on the mirror is observed by the photoelectric cell. A beam of light is directed onto the mirror and reflected into the photodetector cell. The photodetector senses the change in reflectance when condensation occurs on the mirror. The thermocouple attached to the mirror then records the temperature at which condensation (dew) occurs. The dew point temperature is determined by averaging dew point temperatures measured by repeating the cooling and heating cycles of the mirror, which cause dew on the mirror to form and disappear, respectively. Once the temperature measurements are complete and the  $a_w$  calculated, the final  $a_w$  and temperature of the sample are displayed. Additional details regarding the chilled mirror dew point technology,

as well as schematic drawings of the instrument, are given in Campbell and Lewis (1998).

The dew point hygrometer uses an internal fan that circulates the air within the sample chamber to reduce vapour equilibration time, and since both dew point and sample surface temperatures are measured individually, the need for complete thermal equilibrium is eliminated. These features reduce sample measurement times to usually less than 5 min. However, some samples may require longer equilibration times due to their moisture sorption characteristics, such as high fat (e.g., water-in-oil emulsions, such as butter), high viscosity, glassy texture (e.g., extremely dry or dehydrated samples), or multiple component samples, as well as samples that contain a coating(s) (AquaLab Operator's Manual, 2002; Fontana, 2005).

In addition, the same saturated salt slurries, potassium acetate and potassium chloride, were used to verify the linear offset of the dew point hygrometer at the beginning of the experiment. The mean of three readings for both salts at 25 °C were within the reported mean  $a_w$  values for the two salts plus or minus the reported accuracy of the dew point hygrometer ( $\pm 0.003$ , Water Activity Catalog, 2005); therefore adjustment of the linear offset was not necessary. The reported mean  $a_w$  values of saturated potassium acetate and potassium chloride, obtained by averaging four literature values are 0.222 and 0.843 at 25 °C, respectively (Greenspan, 1976; Nyqvist, 1983; Stokes & Robinson, 1949; Young, 1967).

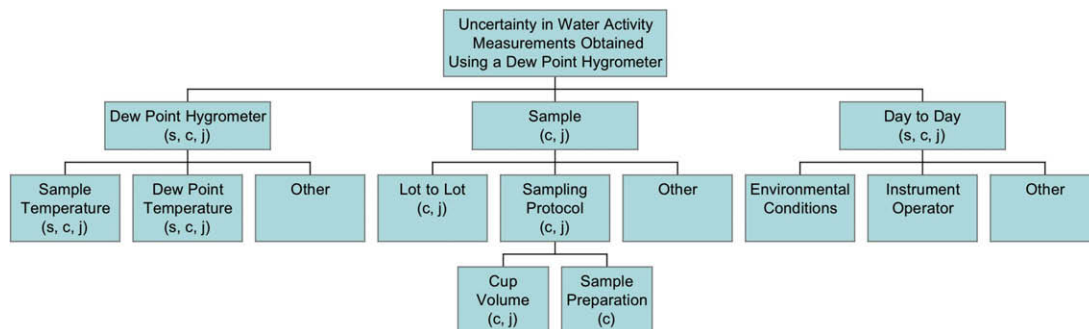
## 2.3. Methods

The dew point hygrometer was used according to the manufacturer's specifications (AquaLab Operator's Manual, 2002) to measure the sample  $a_w$  values. The dew point hygrometer was turned on each day and allowed to warm up for 15–20 min before measurements were taken to allow the chamber temperature to reach the set temperature, 25 °C. Samples were transferred to a sample cup and filled to approximately  $\frac{3}{4}$  full (unless otherwise specified); the sample cup was then placed in the instrument sample drawer. After the drawer was closed, the drawer knob was turned to the READ position to seal the sample cup within the measurement chamber.  $a_w$  values were then obtained for the sample set of interest (sample sets described below) using the continuous mode. The continuous mode setting runs a test to completion, displays the data, and then immediately begins the next test on the same sample without opening the instrument sample drawer. In general, for corn flakes, the first and second continuous mode  $a_w$  readings took approximately 15 and 8 min, respectively, with subsequent  $a_w$  readings in a set taking approximately 5–6 min each. For the saturated salt slurries and jam samples, in general, all  $a_w$  readings within a set took no more than 5 min each. Between sample sets, activated charcoal was run to absorb any residual humidity in the sample chamber.

Ten continuous  $a_w$  readings of the same cup of saturated potassium acetate slurry and potassium chloride slurry at 25 °C were obtained on six and three different days, respectively, to estimate the day to day uncertainty of the  $a_w$  measurement.

Ten continuous  $a_w$  readings of “as is” corn flakes (lot# KMB108 labelled as CF1) on the same sample<sup>1</sup> at 25 °C were obtained on five different days to estimate the day to day uncertainty of the measurement. Ten continuous  $a_w$  readings of three lots of “as is” corn flakes

<sup>1</sup> It is important to note that the same corn flake sample was measured over the 5 days (stored as described in Section 2). A question may arise as to whether using the same sample over the 5 days would result in a change in the moisture content of the sample (since the sample is striving to come to equilibrium within the chamber air during each data set and glassy samples, like corn flakes, may have a challenging time coming to equilibrium without a change in moisture content) that would result in a change in sample  $a_w$ . Based on the day to day  $a_w$  data (see Supplementary material Table C, last readings for each day) no increase in  $a_w$  over the 5 days was observed.



**Fig. 1.** Possible sources of uncertainty associated with dew point hygrometer-obtained  $a_w$  measurements. Lower case letters indicate which sources of uncertainty were considered for each material studied, salt slurries (s), corn flakes (c), and jam (j).

at 25 °C (lot# KMB108 labelled as CF1, lot# KLC003 labelled as CF2, and lot# KMC101 labelled as CF3) were obtained on the same day to estimate the lot to lot uncertainty. Ten continuous  $a_w$  readings of “as is” corn flakes (lot# KMB108 labelled as CF1) at 25 °C were obtained on the same day at three cup volumes,  $\frac{3}{4}$  full cup (3 g),  $\frac{1}{2}$  full cup (2 g), and  $\frac{1}{4}$  full cup (1 g), to estimate the uncertainty from cup volume. Ten continuous  $a_w$  readings of “as is” and ground corn flakes (lot# KMB108 labelled as CF1) at 25 °C were obtained on the same day to estimate the uncertainty from sample preparation.

Ten continuous  $a_w$  readings of jam (lot# 601100102272 labelled as GJ1) at 25 °C were obtained on three different days to estimate the day to day uncertainty of the measurement. Ten continuous  $a_w$  readings of three lots of jam at 25 °C (lot# 601100102272 labelled as GJ1, lot# 53350011028 labelled as GJ2, and lot# 53350011155 labelled as GJ3) were obtained on the same day to estimate the lot to lot uncertainty. Ten continuous  $a_w$  readings of jam (lot# 601100102272 labelled as GJ1) at 25 °C were obtained on the same day at two cup volumes,  $\frac{3}{4}$  full cup (9 g),  $\frac{1}{2}$  full cup (6 g), to estimate the cup volume uncertainty.

#### 2.4. Data analysis procedures

Two data analysis procedures were employed in this study. First, one-way analysis of variance (ANOVA) was applied to each of the material data sets (e.g., corn flakes) to determine whether a source of uncertainty (Fig. 1, e.g., day to day changes) was statistically significant, at  $p = 0.05$ . If the targeted source of uncertainty was determined to be significant then the second procedure was applied to the material data set, uncertainty analysis. Uncertainty analysis provides a quantitative estimate of the contribution of each source of uncertainty to the combined standard uncertainty. However, if a source of uncertainty was determined not to be significant, uncertainty analysis was not carried out on that source of uncertainty. Combining ANOVA with uncertainty analysis provides both a test of statistical significance and a means of estimating the portion of the combined standard uncertainty that can be attributed to each significant source of uncertainty. The one-way ANOVA was carried using the data analysis tools in Microsoft Excel 2004 for the Mac (version 11.3.6). The details of the uncertainty analysis are provided in Section 3.

### 3. Results and discussion

#### 3.1. Identification

Possible sources of uncertainty associated with  $a_w$  measurements using the dew point hygrometer are divided into three major categories (Fig. 1): those associated with the instrument, sample point and dew point temperatures, those associated with the sample, lot to lot and sampling protocol (specifically cup vol-

ume and sample preparation, in this case “as is” versus gentle grinding of corn flakes with a mortar and pestle), and those associated with temporal or day to day changes.<sup>2</sup> Possible sources of day to day uncertainty include, but are not limited to, environmental conditions, such as temperature and relative humidity of the room in which the  $a_w$  measurements were obtained (these were monitored, but not adjusted in this study) and instrument operator (kept constant in this study). Due to the different characteristics associated with each material type, the sources of uncertainty vary from material to material. Thus, the specific sources of uncertainty investigated for each material are identified, using lower case letters [saturated salt slurries (s), corn flakes (c), and jam (j)], in Fig. 1. While it is recognised that there is some natural variability in the saturated salt slurry materials, this should be negligibly small compared to the other sources of uncertainties and therefore was not investigated here.

Other sources of uncertainty (represented by boxes with the term “other” in Fig. 1), in addition to the ones specified above, could be considered for investigation in future studies, for example, the uncertainty associated with the constants used in Eq. (1) (instrument uncertainty), age and storage conditions prior to measurement of the sample (sample uncertainty), and location of the instrument (day to day uncertainty). However, it was decided based on experience and input from others that the sources investigated were some of the more common contributors to uncertainty.

#### 3.2. Specification, quantification and combination

##### 3.2.1. Instrument uncertainty

As described by Campbell and Lewis (1998), the dew point hygrometer calculates the  $a_w$  of a sample using the following equation:

$$a_w = \frac{a \exp\left(\frac{bT_d}{T_d+c}\right)}{a \exp\left(\frac{bT_s}{T_s+c}\right)} = \exp\left(\frac{bc(T_d - T_s)}{(T_d + c)(T_s + c)}\right) \quad (2)$$

where  $T_s$  is the surface temperature of the sample and  $T_d$  is the dew point temperature both in °C,  $a$ ,  $b$ , and  $c$  are all constants ( $a$  equals 0.611 kPa,  $b$  equals  $17.5 \text{ C}^{-1}$  and  $c$  equals 241 °C). When the dew point hygrometer measures the sample  $a_w$ , it measures both  $T_s$  and  $T_d$ . However, upon completion of the measurement only the value of  $T_s$  is displayed, along with the calculated  $a_w$  value. To obtain  $T_d$ , Eq. (2) was rearranged to yield the below equation:

<sup>2</sup> During the course of this study, tests to quantify the reproducibility of the results showed variability in identical experiments performed on different days. This temporal uncertainty is termed day to day uncertainty in Fig. 1. Whether this day to day uncertainty is associated with the sample, the instrument, and/or some other factor was not clear from the results, so this was listed as a third major source of uncertainty in Fig. 1.



**Table 1**  
Day one  $a_w$  readings of saturated potassium acetate slurry at 25 °C obtained over six days (full data set available in the Supplementary Data Table A) and the calculated instrument variance,  $u_i^2(a_w)$ , and uncertainty,  $u_i(a_w)$ , of each  $a_w$  reading.

Continuous readings	$a_w$	$T_s$ (°C)	$T_d$ (°C)	$\partial a_w/\partial T_s$	$\partial a_w/\partial T_d$	$u_i^2(a_w)$	$u_i(a_w)$
Day 1							
1	0.219	25.1	1.832	-0.01304	0.01566	1.704E-07	4.127E-04
2	0.219	25.1	1.832	-0.01304	0.01566	1.704E-07	4.127E-04
3	0.220	25.0	1.812	-0.01311	0.01574	1.720E-07	4.147E-04
4	0.220	25.0	1.812	-0.01311	0.01574	1.720E-07	4.147E-04
5	0.220	25.0	1.812	-0.01311	0.01574	1.720E-07	4.147E-04
6	0.220	25.0	1.812	-0.01311	0.01574	1.720E-07	4.147E-04
7	0.220	25.0	1.812	-0.01311	0.01574	1.720E-07	4.147E-04
8	0.220	25.0	1.812	-0.01311	0.01574	1.720E-07	4.147E-04
9	0.220	25.0	1.812	-0.01311	0.01574	1.720E-07	4.147E-04
10	0.220	25.0	1.812	-0.01311	0.01574	1.720E-07	4.147E-04

$$\frac{bT_d}{(T_d + c)} = \ln a_w + \frac{bT_s}{c + T_s} \quad (3)$$

Based on Eq. (2), the  $a_w$  measurements are fully dependent on  $T_s$  and  $T_d$  readings, so the variance in  $a_w$  can be determined by differentiating Eq. (2) with respect to  $T_s$  and to  $T_d$  resulting in Eqs. (4) and (5), respectively:

$$\partial a_w/\partial T_s = -a_w \cdot b \cdot c/(T_s + c)^2 \quad (4)$$

$$\partial a_w/\partial T_d = a_w \cdot b \cdot c/(T_d + c)^2 \quad (5)$$

The variance of  $a_w$  from the uncertainty of  $T_s$  and  $T_d$  [instrument variance,<sup>3</sup>  $u_i^2(a_w)$ ] is calculated by combining both  $T_s$  and  $T_d$  variances, as shown in the following equation:

$$u_i^2(a_w) = \left(\frac{\partial a_w}{\partial T_s}\right)^2 u^2(T_s) + \left(\frac{\partial a_w}{\partial T_d}\right)^2 u^2(T_d) \quad (6)$$

The standard deviation of standard deviation of  $T_s$  is  $\pm 0.01$  °C and  $T_d$  is  $\pm 0.025$  °C (obtained from the manufacture), therefore  $u^2(T_s)$  and  $u^2(T_d)$  are 0.0001 and 0.000625, respectively.

The first  $a_w$  reading of the potassium acetate slurry on day 1, 0.219  $a_w$  at 25 °C (Table 1, day 1), is used below as an example to illustrate the calculation of the instrument variance [ $u_i^2(a_w)$ ] and uncertainty [standard deviation,  $u_i(a_w)$ ].

When  $a_w = 0.219$  at  $T_s = 25.1$  °C,  $T_d$  is calculated as follows:

$$\frac{bT_d}{(T_d + c)} = \ln 0.219 + \frac{bT_s}{c + T_s} = 0.132 \quad (\text{using Eq.(3)})$$

Solving for  $T_d$  yields

$$T_d = \frac{0.132 * 241}{17.5 - 0.132} = 1.83 \text{ °C}$$

Then the partial derivatives of  $a_w$  with respect to  $T_s$  and to  $T_d$  were calculated:

$$\partial a_w/\partial T_s = -0.219 * 17.5 * 241/(25.1 + 241)^2 = -0.01304 \quad (\text{using Eq.(4)})$$

$$\partial a_w/\partial T_d = 0.219 * 17.5 * 241/(1.83 + 241)^2 = 0.01566 \quad (\text{using Eq.(5)})$$

and combined to yield:

$$u_i^2(a_w) = (-0.013)^2 * 0.0001 + 0.016^2 * 0.000625 = 1.704E - 07 \quad (\text{using Eq.(6)})$$

and the combined standard uncertainty:

$$u_i(a_w) = \sqrt{1.704E - 07} = 4.127E - 04$$

The percentage of the  $u_i^2(a_w)$  from  $T_s$  was calculated by dividing the variance contributed from  $T_s$  by  $u_i^2(a_w)$ , and, on average, ranged between 10% and 13% across all material types studied. The percentage of  $u_i^2(a_w)$  from  $T_d$  was calculated by dividing the variance contributed from  $T_d$  by  $u_i^2(a_w)$ , and, on average, ranged between 90% and 87% across all material types studied.

The measured  $a_w$  readings and calculated instrument uncertainties for the sources of uncertainty identified in Fig. 1 for each material are available as Supplementary material (Tables A–I).

### 3.2.2. Other sources of uncertainty

The one-way ANOVA results for each material for each source of uncertainty (Fig. 1) are given in Table 2. Uncertainty analysis was then applied to the sources of uncertainty that were determined to be statistically significant, at  $p = 0.05$ . The day to day source of uncertainty for both potassium chloride and jam materials was found not to be significant.<sup>4</sup>

The influence of each significant source of uncertainty was quantified using the inverse combination method mentioned in the Introduction. Statically determined uncertainty (Type A, ISO, 1995) from all the experimental measurements within a data set was treated as the overall combined uncertainty,  $u_c$ . Similarly, Type A uncertainty for each treatment within a data set was determined based on the experimental measurements, where the targeted source of uncertainty was excluded. Eq. (1) was then used to determine the resulting portion of the overall combined uncertainty that was attributable to the targeted source of uncertainty. For example, to estimate the day to day uncertainty,  $u_d$ , 10 replicate  $a_w$  measurements were made in a single day on the same sample. The mean of those 10 measurements was treated as the  $a_w$  value for that day's experiment and the standard deviation of those 10 measurements was the uncertainty from all sources of uncertainty except the day to day uncertainty,  $u_c$ . This was repeated on  $N$  subsequent days, using the same sample. Next, the overall combined uncertainty,  $u_c$ , which reflects both measurement and day to day uncertainties, was determined from the daily mean  $a_w$  values for all  $N$  days. Lastly, the combined variance excluding the targeted source,  $u_c^2$ , was calculated and subtracted from the overall combined variance,  $u_d^2$ , via Eq. (7), yielding an estimate of the day to day variance,  $u_d^2$ , and the day to day uncertainty,  $u_d$ .

$$u_d^2 = u_c^2 - \frac{1}{N^2} \sum_{i=1}^N u_{c_i}^2 \quad (7)$$

<sup>3</sup> The full notation for the variance and uncertainty of  $a_w$  in this manuscript is  $u_{\text{subscript}}^2(a_w)$  and  $u_{\text{subscript}}(a_w)$ , respectively, as used here for the instrument variance. However, in cases where only the uncertainty of  $a_w$  is involved the variance and uncertainty full notations are simplified to  $u_{\text{subscript}}^2$  and  $u_{\text{subscript}}$ , respectively.

<sup>4</sup> It is interesting to note that for the potassium chloride and jam materials, the variance of the daily means was smaller than the variance within a day, so a day to day variance could not be calculated, which is consistent with the one-way ANOVA results (Table 2) where day to day was not a significant source of uncertainty for these two materials.

**Table 2**

One-way ANOVA results for each material for each source of uncertainty investigated, as outlined in Fig. 1. *p*-Values less than 0.05 indicate that the source of uncertainty was significant and uncertainty analysis was carried out.

Material	Source of variation			
	Day to day	Lot to lot	Cup volume	Sample preparation
Potassium acetate	<0.0001	NA <sup>a</sup>	NA	NA
Potassium chloride	0.5366	NA	NA	NA
Corn flakes	<0.0001	<0.0001	<0.0001	<0.0001
Grape jam	0.0971	<0.0001	0.0373	NA

<sup>a</sup> NA indicates that the source of uncertainty was not assessed for that material.

**Table 3**

Statistical analysis of  $a_w$  readings (located in Supplementary material Table A) of the saturated potassium acetate slurry at 25 °C over day 1–day 6.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6		Day to day
Daily mean	0.2198	0.2204	0.2213	0.2224	0.2214	0.2193	Overall day to day mean	0.2208
Measurement uncertainty, $u_m$	4.216E–04	5.164E–04	4.831E–04	5.164E–04	5.164E–04	4.831E–04	Overall combined uncertainty, $u_c$	1.147E–03
Measurement variance, $u_m^2$	1.778E–07	2.667E–07	2.333E–07	2.667E–07	2.667E–07	2.333E–07	Overall combined variance, $u_c^2$	1.315E–06
Instrument uncertainty, $u_i$	1.310E–04	1.315E–04	1.321E–04	1.325E–04	1.319E–04	1.309E–04	Combined uncertainty excluding targeted source, $u_e$	2.003E–04
Instrument variance, $u_i^2$	1.717E–08	1.730E–08	1.745E–08	1.757E–08	1.740E–08	1.713E–08	Combined variance excluding targeted source, $u_e^2$	4.012E–08
Other uncertainty, $u_o$	4.007E–04	4.994E–04	4.647E–04	4.991E–04	4.993E–04	4.650E–04	Day to day uncertainty, $u_d$	1.129E–03
Other variance, $u_o^2$	1.606E–07	2.494E–07	2.159E–07	2.491E–07	2.493E–07	2.162E–07	Day to day variance, $u_d^2$	1.275E–06
$\Omega$ Measurement	0.19%	0.23%	0.22%	0.23%	0.23%	0.22%	$\Omega$ Day to day	0.51%
$\Omega$ Instrument	0.06%	0.06%	0.06%	0.06%	0.06%	0.06%		
$\Omega$ Other	0.18%	0.23%	0.21%	0.22%	0.23%	0.21%		
%Var instrument <sup>a</sup>	9.66%	6.49%	7.48%	6.59%	6.53%	7.34%		
%Var other <sup>b</sup>	90.34%	93.51%	92.52%	93.41%	93.47%	92.66%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.

<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.

#### 4. Uncertainty analysis results

The summary of the uncertainty analysis of dew point hygrometer-obtained  $a_w$  readings for the saturated potassium acetate slurry at 25 °C is shown in Table 3 (measurement, instrument, and day to day). The summary of the uncertainty analysis of  $a_w$  readings for the saturated potassium chloride slurry at 25 °C is shown in Table 4 (measurement and instrument). The summary of the uncertainty analysis of  $a_w$  readings for corn flakes at 25 °C is shown in Tables 5 (measurement, instrument, and day to day), 6 (measurement, instrument, and lot to lot), 7 (measurement, instrument, and sample preparation), and 8 (measurement, instrument, and cup volume). The uncertainty analysis of  $a_w$  readings for jam at 25 °C is shown in Tables 9 (measurement and instrument), 10 (measurement, instrument, and lot to lot) and 11 (measurement, instrument, and cup volume). The coefficient of variation ( $\Omega$ , Omega), which is the ratio of the uncertainty divided by the mean, was calculated for each source of uncertainty for each material. Use of  $\Omega$  facilitates comparison of the magnitude of uncertainties for materials with different mean values.

$\Omega$  Values for all four materials from all tested sources of uncertainties (instrument, day to day, lot to lot, cup volume, and sample preparation) are plotted in Fig. 2, allowing comparison of the contribution of each uncertainty source to be compared among materials. Overall, it can be observed that the instrument  $\Omega$  values are very small and consistent among all four materials, which indicates that the performance of the tested dew point hygrometer is precise and stable. Other uncertainty highlights from Fig. 2 are discussed below.

Among the four materials, corn flakes showed the largest  $\Omega$  for all tested uncertainty sources. This is probably due to the glassy, case-hardened nature of the corn flake sample. A consistent decrease followed by a leveling off in corn flake  $a_w$  values was ob-

served for every set of corn flake  $a_w$  data obtained (see Supplementary material Tables C–F). To further investigate this decreasing  $a_w$  behaviour additional data were collected. These additional data were not included in the original uncertainty analysis calculations, but rather used to explore how to reduce  $a_w$  measurement uncertainty and optimise  $a_w$  measurement procedures (objective 2) for the corn flake sample. Thirty-five additional  $a_w$  data points were collected in the continuous mode on “as is” corn flakes (lot CF1) and, for comparison purposes, on potassium acetate (Fig. 3). As observed in Fig. 3,  $a_w$  readings of potassium acetate stabilised within six readings,<sup>5</sup> while  $a_w$  readings of “as is” corn flakes continued to decrease until about reading number 30.

When measuring the  $a_w$  of corn flakes under normal ambient conditions (average ambient %RH of 30–40%), the corn flakes would need to gain moisture from the air to both dominate and equilibrate with the sealed sample chamber headspace. Reaching this equilibrium for the corn flake samples took a rather long time – approximately 180 min (time to reach reading number 30). This extensive equilibration time is hypothesised to be due to the glassy state<sup>6</sup> of the corn flakes at the experimental temperature, 25 °C. For the corn flake sample there are probably two equilibration times of significance to the  $a_w$  measurement: (1) the equilibration of the sample surface with the chamber air (where the sample  $a_w$  is intended to dominate this equilibration) and (2) the equilibration of the sample surface with the rest of the corn flake sample, which are of variable thicknesses. For many material types (e.g., the salt slurries and jam

<sup>5</sup> The fluctuation of  $a_w$  readings of potassium acetate slurry at the beginning of the continuous readings was mainly attributed to the slight increase in the temperature of the sample as it was attempting to equilibrate to the target temperature.

<sup>6</sup> The  $T_g$  of an extruded corn flour and starch-zein based material (similar to the corn flake) was determined to be 85 °C using differential scanning calorimetry by Chanvrier, Colonna, Valle, and Lourdin (2004).

**Table 4**Statistical analysis of  $a_w$  readings (located in Supplementary Data Table B) of the saturated potassium chloride slurry at 25 °C over day 1–day 3.

	Day 1	Day 2	Day 3		Day to day
Daily mean	0.8441	0.8447	0.8450	Overall day to day mean	0.8446
Measurement uncertainty, $u_m$	1.197E–03	1.889E–03	2.211E–03	Overall combined uncertainty, $u_c$	4.583E–04
Measurement variance, $u_m^2$	1.433E–06	3.567E–06	4.889E–06	Overall combined variance, $u_c^2$	2.100E–07
Instrument uncertainty, $u_i$	4.380E–04	4.388E–04	4.390E–04	Combined uncertainty excluding targeted source, $u_e$	1.048E–03
Instrument variance, $u_i^2$	1.919E–07	1.926E–07	1.928E–07	Combined variance excluding targeted source, $u_e^2$	1.099E–06
Other uncertainty, $u_o$	1.114E–03	1.837E–03	2.167E–03		
Other variance, $u_o^2$	1.242E–06	3.374E–06	4.696E–06		
$\Omega$ Measurement	0.14%	0.22%	0.26%		
$\Omega$ Instrument	0.05%	0.05%	0.05%		
$\Omega$ Other	0.13%	0.22%	0.26%		
%Var instrument <sup>a</sup>	13.39%	5.40%	3.94%		
%Var other <sup>b</sup>	86.61%	94.60%	96.06%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.**Table 5**Statistical analysis of  $a_w$  readings (located in Supplementary Data Table C) of “as is” Kellogg’s corn flakes (lot CF1) at 25 °C over day 1–day 5.

	Day 1	Day 2	Day 3	Day 4	Day 5		Day to day
Daily mean	0.1990	0.1995	0.1993	0.1912	0.2045	Overall day to day mean	0.1987
Measurement uncertainty, $u_m$	2.494E–03	7.071E–04	1.767E–03	1.619E–03	8.498E–04	Overall combined uncertainty, $u_c$	4.769E–03
Measurement variance, $u_m^2$	6.222E–06	5.000E–07	3.122E–06	2.622E–06	7.222E–07	Overall combined variance, $u_c^2$	2.275E–05
Instrument uncertainty, $u_i$	1.199E–04	1.201E–04	1.201E–04	1.158E–04	1.228E–04	Combined uncertainty excluding targeted source, $u_e$	7.264E–04
Instrument variance, $u_i^2$	1.437E–08	1.443E–08	1.441E–08	1.340E–08	1.509E–08	Combined variance excluding targeted source, $u_e^2$	5.276E–07
Other uncertainty, $u_o$	2.492E–03	6.968E–04	1.763E–03	1.615E–03	8.409E–04	Day to day uncertainty, $u_d$	4.714E–03
Other variance, $u_o^2$	6.208E–06	4.856E–07	3.108E–06	2.609E–06	7.071E–07	Day to day variance, $u_d^2$	2.222E–05
$\Omega$ Measurement	1.25%	0.35%	0.89%	0.85%	0.42%	$\Omega$ Day to day	2.37%
$\Omega$ Instrument	0.06%	0.06%	0.06%	0.06%	0.06%		
$\Omega$ Other	1.25%	0.35%	0.88%	0.84%	0.41%		
%Var instrument <sup>a</sup>	0.23%	2.89%	0.46%	0.51%	2.09%		
%Var other <sup>b</sup>	99.77%	97.11%	99.54%	99.49%	97.91%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.**Table 6**Statistical analysis of  $a_w$  readings (located in Supplementary Data Table D) of three lots of “as is” Kellogg’s corn flakes (lot CF1, lot CF2, and lot CF3) at 25 °C.

	Lot CF1	Lot CF2	Lot CF3		Lot to lot
Lot mean	0.199	0.2112	0.1923	Overall lot to lot mean	0.2008
Measurement uncertainty, $u_m$	2.494E–03	2.394E–03	2.946E–03	Overall combined uncertainty, $u_c$	9.582E–03
Measurement variance, $u_m^2$	6.222E–06	5.733E–06	8.678E–06	Overall combined uncertainty variance, $u_c^2$	9.182E–05
Instrument uncertainty, $u_i$	1.199E–04	1.264E–04	1.163E–04	Combined uncertainty excluding targeted source, $u_e$	1.514E–03
Instrument variance, $u_i^2$	1.437E–08	1.599E–08	1.351E–08	Combined variance excluding targeted source, $u_e^2$	2.293E–06
Other uncertainty, $u_o$	2.492E–03	2.391E–03	2.943E–03	Lot to lot uncertainty, $u_l$	9.462E–03
Other variance, $u_o^2$	6.208E–06	5.717E–06	8.664E–06	Lot to lot variance, $u_l^2$	8.953E–05
$\Omega$ Measurement	1.25%	1.13%	1.53%	$\Omega$ Lot to lot	4.71%
$\Omega$ Instrument	0.06%	0.06%	0.06%		
$\Omega$ Other	1.25%	1.13%	1.53%		
%Var instrument <sup>a</sup>	0.23%	0.28%	0.16%		
%Var other <sup>b</sup>	99.77%	99.72%	99.84%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.

materials tested here), these two equilibrations are achieved rather quickly and over similar time frames. However, for corn flakes, which are both glassy and case-hardened, it appears that the first equilibrium is achieved, which allows for an  $a_w$  measurement to be completed and reported by the instrument (~15 min), but that the continuous decrease in  $a_w$  followed by a leveling off is due to the contribution of the second equilibrium. Thus, the contribution of surface sorption of water from the chamber air followed by slow diffusion into and within the glassy corn flake material resulted in the observed decrease in  $a_w$  over time (reading number). It would

be interesting to compare the continuous mode  $a_w$  readings over time (reading number) of other types of glassy materials to determine if they exhibit a similar  $a_w$  equilibration pattern or if other sample features (e.g., composition, particle size, and processing methods) serve to influence (e.g., speed up or slow down) equilibration.

The corn flake data serves as a rather vivid illustration of why longer equilibration times are required for glassy materials and points to data collection duration (# of continuous  $a_w$  readings) as another important factor that influences the uncertainty of

**Table 7**Statistical analysis of  $a_w$  readings (located in Supplementary Data Table E) of “as is” and ground Kellogg’s corn flakes (lot CF1) at 25 °C.

	“as is”	Ground		Sample preparation
Sample preparation mean	0.1960	0.1900	Overall sample preparation mean	0.1930
Measurement uncertainty, $u_m$	2.357E–03	1.636E–03	Overall combined uncertainty, $u_c$	4.455E–03
Measurement variance, $u_o$	5.556E–06	2.678E–06	Overall combined variance, $u_c^2$	1.985E–05
Instrument uncertainty, $u_i$	1.184E–04	1.149E–04	Combined uncertainty excluding targeted source, $u_e$	1.435E–03
Instrument variance, $u_i^2$	1.401E–08	1.321E–08	Combined variance excluding targeted source, $u_e^2$	2.058E–06
Other uncertainty, $u_o$	2.354E–03	1.632E–03	Sample preparation uncertainty, $u_s$	4.217E–03
Other variance, $u_o^2$	5.542E–06	2.665E–06	Sample preparation variance, $u_s^2$	1.779E–05
$\Omega$ Measurement	1.20%	0.86%	$\Omega$ Sample preparation	2.19%
$\Omega$ Instrument	0.06%	0.06%		
$\Omega$ Other	1.20%	0.86%		
%Var instrument <sup>a</sup>	0.25%	0.49%		
%Var other <sup>b</sup>	99.75%	99.51%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.**Table 8**Statistical analysis of  $a_w$  readings (located in Supplementary Data Table F) of “as is” Kellogg’s corn flakes (lot CF1) at 25 °C for three cup volumes.

	$\frac{1}{4}$ Full	$\frac{1}{2}$ Full	$\frac{3}{4}$ Full		Cup volume
Cup volume mean	0.1912	0.1842	0.1767	Overall cup volume mean	0.1840
Measurement uncertainty, $u_m$	1.619E–03	2.300E–03	3.773E–03	Overall combined uncertainty, $u_c$	7.251E–03
Measurement variance, $u_o$	2.622E–06	5.289E–06	1.423E–05	Overall combined variance, $u_c^2$	5.258E–05
Instrument uncertainty, $u_i$	1.157E–04	1.119E–04	1.078E–04	Combined uncertainty excluding targeted source, $u_e$	1.568E–03
Instrument variance, $u_i^2$	1.340E–08	1.253E–08	1.163E–08	Combined variance excluding targeted source, $u_e^2$	2.460E–06
Other uncertainty, $u_o$	1.615E–03	2.297E–03	3.771E–03	Cup volume uncertainty, $u_v$	7.080E–03
Other variance, $u_o^2$	2.609E–06	5.276E–06	1.422E–05	Cup volume variance, $u_v^2$	5.012E–05
$\Omega$ Measurement	0.85%	1.25%	2.14%	$\Omega$ Cup volume	3.85%
$\Omega$ Instrument	0.06%	0.06%	0.06%		
$\Omega$ Other	0.84%	1.25%	2.13%		
%Var instrument <sup>a</sup>	0.51%	0.24%	0.08%		
%Var other <sup>b</sup>	99.49%	99.76%	99.92%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.**Table 9**Statistical analysis of  $a_w$  readings (located in Supplementary Data Table G) of Smucker’s Concord grape jam (lot GJ1) at 25 °C from day 1 to day 3.

	Day 1	Day 2	Day 3		Day to day
Daily mean	0.8393	0.8378	0.8379	Overall day to day mean	0.8383
Measurement uncertainty, $u_m$	1.567E–03	1.687E–03	1.729E–03	Overall combined uncertainty, $u_c$	8.387E–04
Measurement variance, $u_o^2$	2.456E–06	2.844E–06	2.989E–06	Overall combined variance, $u_c^2$	7.033E–07
Instrument uncertainty, $u_i$	4.361E–04	4.341E–04	4.337E–04	Combined uncertainty excluding targeted source, $u_e$	9.597E–04
Instrument variance, $u_i^2$	1.902E–07	1.884E–07	1.881E–07	Combined variance excluding targeted source, $u_e^2$	9.210E–07
Other uncertainty, $u_o$	1.505E–03	1.630E–03	1.674E–03		
Other variance, $u_o^2$	2.265E–06	2.656E–06	2.801E–06		
$\Omega$ Measurement	0.19%	0.20%	0.21%		
$\Omega$ Instrument	0.05%	0.05%	0.05%		
$\Omega$ Other	0.18%	0.19%	0.20%		
%Var instrument <sup>a</sup>	7.75%	6.62%	6.29%		
%Var other <sup>b</sup>	92.25%	93.38%	93.71%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.

the corn flake  $a_w$  measurements. To further investigate the effects of data collection duration, the experiments examining the effect of sample preparation and cup volume were repeated for a longer duration. Thirty-five  $a_w$  readings of “as is” and ground corn flakes (lot CF1) were collected in the continuous mode (Fig. 4). The initial  $a_w$  readings of “as is” corn flakes are higher than for ground corn flakes. This difference may be due to differences in surface area and/or possibly a decrease in moisture content due to grinding of the sample (although the grinding done with a mortar and pestle was relatively mild). Ground corn flakes have a much larger surface area than “as is” corn flakes, which would facilitate

quicker moisture sorption and diffusion of water into the glassy corn flake matrix. As can be observed in Fig. 4, the difference in  $a_w$  values between “as is” and ground corn flakes decreased as additional continuous mode  $a_w$  readings were collected. Eventually both samples came to a similar  $a_w$  value, which points to surface area effects, rather than a moisture content difference as the cause of the initial  $a_w$  difference. Fig. 4 illustrates that data collection duration has an effect on the uncertainty attributed to sample preparation. Collecting more sample readings (10 versus 35) resulted in a decrease in the  $\Omega$  attributed to sample preparation, from 2.19% to 0.99%.

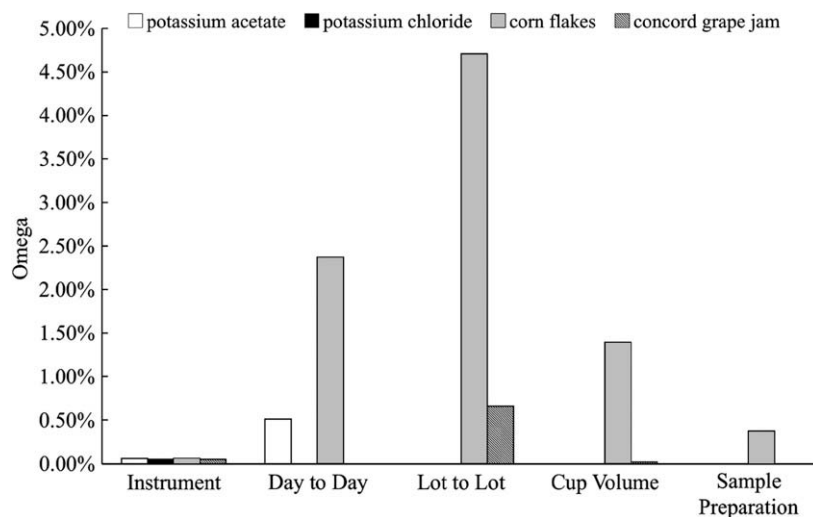


**Table 10**Statistical analysis of  $a_w$  readings (located in Supplementary Data Table H) of three lots of Smucker's Concord grape jam (lot GJ1, lot GJ2, and lot GJ3) at 25 °C.

	Lot GJ1	Lot GJ2	Lot GJ3		Lot to lot
Lot mean	0.8393	0.8286	0.8314	Overall lot to lot mean	0.8331
Measurement uncertainty, $u_m$	1.567E-03	1.838E-03	1.578E-03	Overall combined uncertainty, $u_c$	5.549E-03
Measurement variance, $u_m^2$	2.456E-06	3.378E-06	2.489E-06	Overall combined variance, $u_c^2$	3.079E-05
Instrument uncertainty, $u_i$	4.361E-04	4.306E-04	4.328E-04	Combined uncertainty excluding targeted source, $u_e$	9.616E-04
Instrument variance, $u_i^2$	1.902E-07	1.854E-07	1.873E-07	Combined variance excluding targeted source, $u_e^2$	9.247E-07
Other uncertainty, $u_o$	1.505E-03	1.787E-03	1.517E-03	Lot to lot uncertainty, $u_l$	5.465E-03
Other variance, $u_o^2$	2.265E-06	3.192E-06	2.302E-06	Lot to lot variance, $u_l^2$	2.987E-05
$\Omega$ Measurement	0.19%	0.22%	0.19%	$\Omega$ Lot to lot	0.66%
$\Omega$ Instrument	0.05%	0.05%	0.05%		
$\Omega$ Other	0.18%	0.22%	0.18%		
%Var instrument <sup>a</sup>	7.75%	5.49%	7.53%		
%Var other <sup>b</sup>	92.25%	94.51%	92.47%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.**Table 11**Statistical analysis of  $a_w$  readings (located in Supplementary Data Table I) Smucker's Concord grape jam (lot GJ1) at 25 °C for two cup volumes.

	3/4 Full	1/2 Full		Cup volume
Cup volume mean	0.8379	0.8354	Overall cup volume mean	0.8367
Measurement uncertainty, $u_m$	1.729E-03	3.062E-03	Overall combined uncertainty, $u_c$	1.768E-03
Measurement variance, $u_m^2$	2.989E-06	9.378E-06	Overall combined variance, $u_c^2$	3.125E-06
Instrument uncertainty, $u_i$	4.337E-04	4.327E-04	Combined uncertainty excluding targeted source, $u_e$	1.758E-03
Instrument variance, $u_i^2$	1.881E-07	1.872E-07	Combined variance excluding targeted source, $u_e^2$	3.092E-06
Other uncertainty, $u_o$	1.674E-03	3.0317E-03	Cup volume uncertainty, $u_v$	1.826E-04
Other variance, $u_o^2$	2.801E-06	9.191E-06	Cup volume variance, $u_v^2$	3.333E-08
$\Omega$ Measurement	0.21%	0.37%	$\Omega$ Cup volume	0.02%
$\Omega$ Instrument	0.05%	0.05%		
$\Omega$ Other	0.20%	0.36%		
%Var instrument <sup>a</sup>	6.29%	2.00%		
%Var other <sup>b</sup>	93.71%	98.00%		

<sup>a</sup> Percent variance in  $a_w$  from instrument uncertainty.<sup>b</sup> Percent variance in  $a_w$  from other uncertainty.**Fig. 2.** Contribution of each identified source of uncertainty (Fig. 1) to the overall combined uncertainty of the saturated potassium acetate slurry, saturated potassium chloride slurry, corn flakes, and jam  $a_w$  measurements at 25 °C, expressed as the coefficient of variation ( $\Omega$ , Omega). As discussed in the text, the day to day uncertainty for potassium chloride and jam were found not be statistically significant (Table 2) and thus uncertainty analysis was not carried out.

On the other hand, when 20  $a_w$  readings of three different sample cup volumes of ground corn flakes (lot# NLI00593 labelled as CF4) were collected in the continuous mode (Fig. 5), the initial  $a_w$  readings were very close, but diverged as additional readings were collected. Each corn flake sample appeared to approach an equilibrium  $a_w$  value over time, but the final measured  $a_w$  values from each cup volume were different. This difference in the final  $a_w$  val-

ues is hypothesised to be due to the different capacities of the three sample volumes to dominate and equilibrate with the chamber headspace. It is important to note that the differences in  $a_w$  for the three sample cup volumes represent very small differences in moisture content. Corn flakes have been shown to exhibit a type III (or "J shaped") isotherm in the 0.06–0.55  $a_w$  range, where a very small change in moisture content results in a large change in  $a_w$

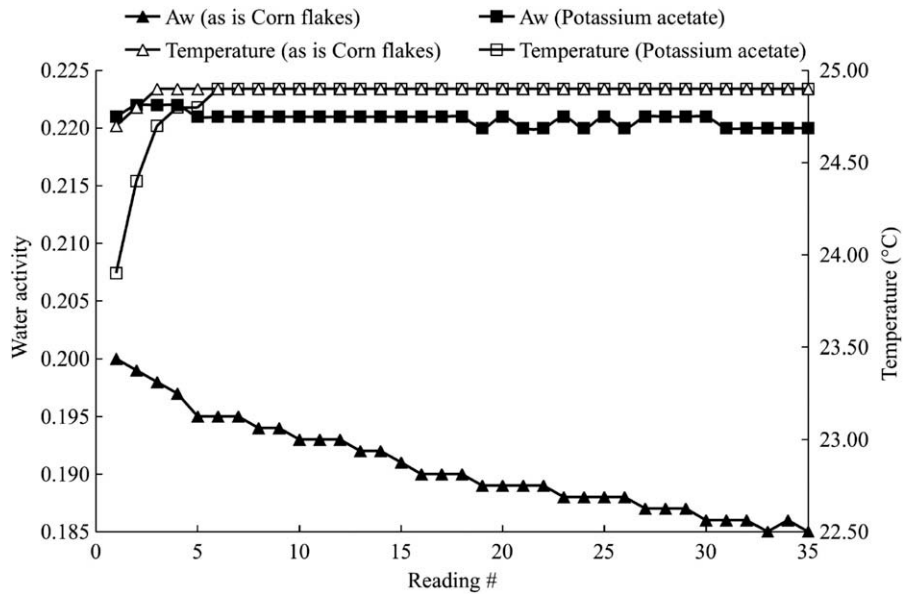


Fig. 3. Thirty-five continuous readings of  $a_w$  and temperature on “as is” corn flake (lot CF1) and saturated potassium acetate slurry materials at 25 °C.

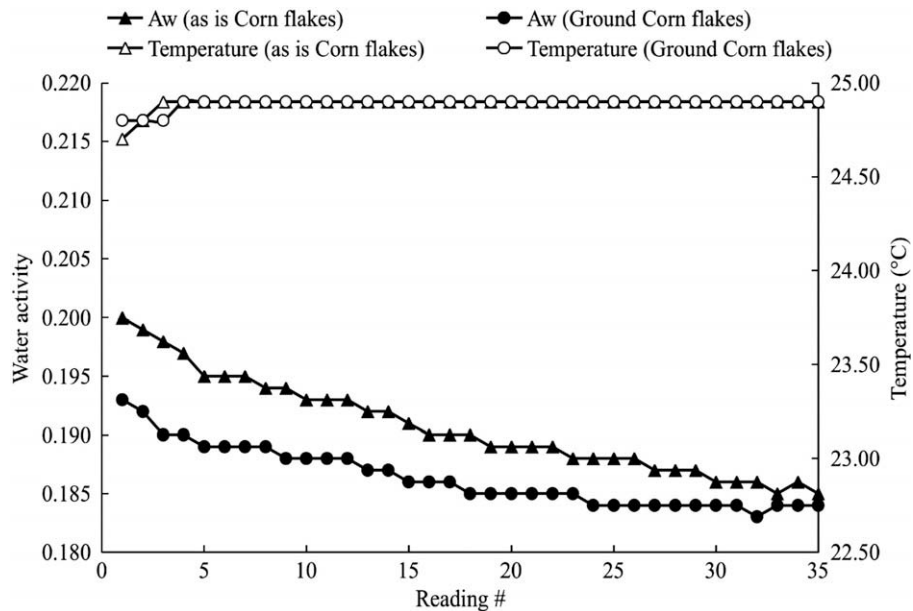


Fig. 4. Thirty-five continuous readings of  $a_w$  and temperature on “as is” and ground corn flakes (lot CF1) at 25 °C.

(Yu, 2007). This “J shaped” behaviour is similar to isotherms of crystalline materials, such as crystalline sucrose, where water adsorbs on the surface of the crystalline sucrose at  $a_w$  values below the sucrose deliquesces point. Thus, corn flakes are not only glassy in nature, but also appear to adsorb water like a crystalline material. However, it is hypothesised that the case-hardened surface of the corn flakes is the structural feature that is responsible for the observed surface adsorption behaviour, not necessarily crystalline structures. The  $a_w$  of materials that participate in surface adsorption are strongly influenced by the relative humidity of the air; however, even large changes in  $a_w$  are actually brought about by rather small changes in moisture content. This may also explain the somewhat higher  $a_w$  values (~0.240–0.225) for corn flakes in Fig. 5, done in the month of May with a higher average ambient

%RH, compared to those in Tables C–F, done in January with a lower average ambient %RH. However, it is also important to note that the corn flakes used in Fig. 5 were from a different lot (lot CF4), which could also be responsible for some of the observed difference in  $a_w$  values.

Richard and Labuza (1990) investigated the influence of cup volume on chilled mirror instrument (CX-1, Decagon Devices, Pullman, WA) response time when measuring the  $a_w$  of two reference solutions (distilled water and a 12% NaCl solution). In general, their data showed an increase in sample  $a_w$  and a decrease in equilibration time as sample volume increased from 1 to 8 ml. These researchers suggested that a minimum of 3 ml of sample be used to speed instrument response time and no more than 6 ml be used to avoid spilling and contamination of the mirror. No explanation

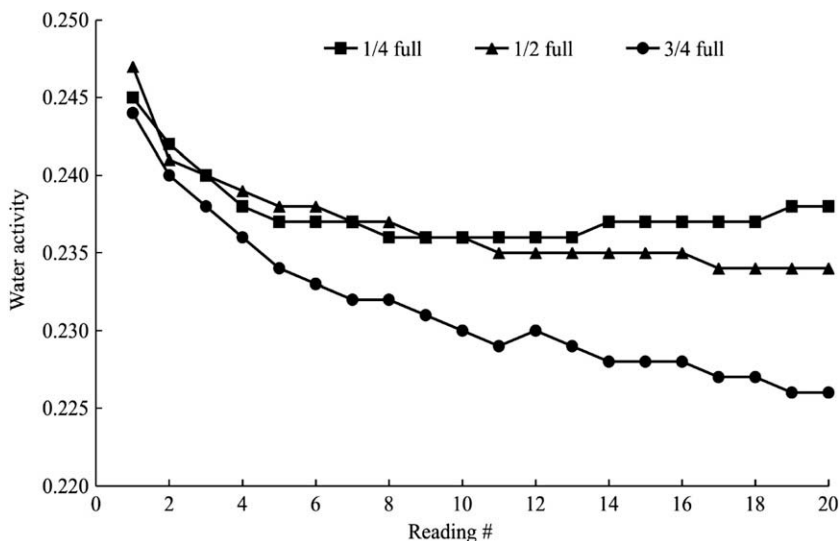


Fig. 5. Twenty continuous readings of  $a_w$  and temperature on three cup volumes of ground corn flakes (lot CF4) at 25 °C.

was offered for the observed increase in  $a_w$  with increasing sample volume.

These sample cup volume results are in concert with the dew point hygrometer manufacturers' suggestion that the sample cup be half full, but, as suggested, not be over filled so as to contaminate the sensor and sensor chamber (AquaLab Operator's Manual, 2002). As specified in the operator's manual, there needs to be enough sample in the cup to allow the water in the sample to equilibrate with the water vapour present in the sample chamber, while not altering the moisture content of the sample. These results also suggest that sample cup volume be optimised and standardised for each material type.

For the jam, the largest uncertainty ( $\Omega = 0.66\%$ ) was due to lot to lot uncertainty, but compared to the corn flake lot to lot uncertainty ( $\Omega = 4.71\%$ ) it was relatively small. The nature of the jam sample, homogenous, relatively high-moisture, continuous material, resulted in less overall uncertainty, compared, for example,

to the corn flake material, a heterogenous, low-moisture (glassy), particulate material.

As previously mentioned, for potassium chloride slurry and jam materials the day to day source of uncertainty was found not to be significant (Table 2). Inspecting the day to day data (Supplementary material Tables B and G) revealed higher than expected  $a_w$  uncertainty within each day. Possible causes for this high within day uncertainty were investigated by plotting the  $a_w$  readings for one day (lowest starting temperature day was selected), along with the corresponding temperature readings, for both potassium chloride slurry (see Supplementary material Table B, day 3) and jam (see Supplementary material Table H, lot CGJ2) (Fig. 6). As can be seen from Fig. 6, it took six readings for the temperature to stabilise when measuring the  $a_w$  of the saturated potassium chloride slurry and jam. The samples were removed from the constant temperature chamber (25 °C) and exposed to ambient lab conditions, approximately  $23 \pm 0.5$  °C, before being placed into the instrument

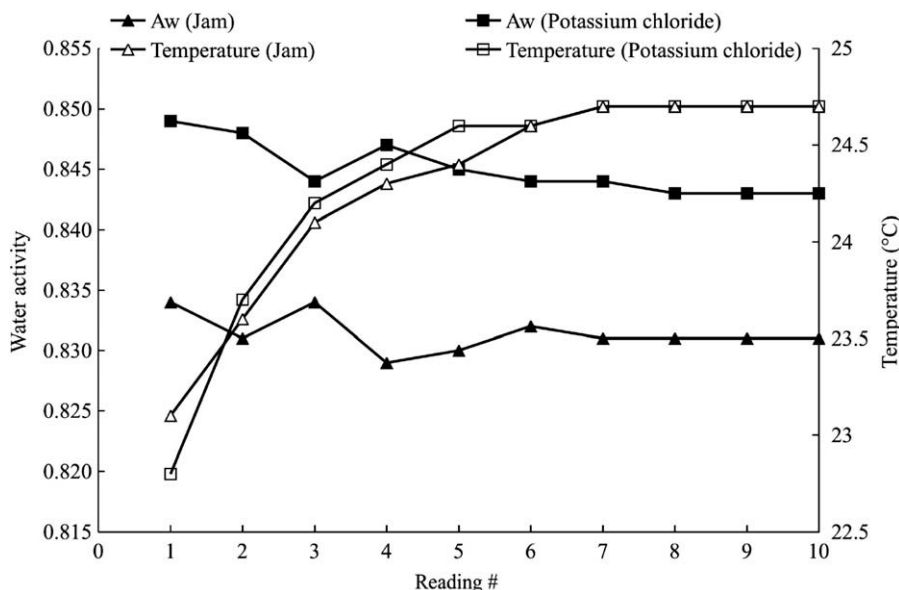


Fig. 6. Ten continuous readings of  $a_w$  and temperature of potassium chloride slurry (Supplementary Data Table B, day 3) and jam (Supplementary Data Table H, lot GJ3) materials at 25 °C.

**Table 12**Overall mean  $a_w$ , overall uncertainty, and overall instrument uncertainty of all four materials, where  $n$  is the number of samples.

Material type and sample number ( $n$ )	Overall mean $a_w$	Overall uncertainty	Overall instrument uncertainty
Potassium acetate, $n = 60$	0.221	$\pm 0.0012$	$\pm 0.0001$
Potassium chloride, $n = 30$	0.845	$\pm 0.0018$	$\pm 0.0003$
Corn flakes, $n = 110$	0.195	$\pm 0.0094$	$\pm 0.00004$
Concord grape jam, $n = 70$	0.835	$\pm 0.0042$	$\pm 0.0002$

sample chamber for measurement. As can be seen in Fig. 6, the initial sample temperatures were around 2 °C lower than the measurement temperature, 25 °C. In high  $a_w$  samples, moisture contents are generally high, and heat capacity values of water large, therefore it takes a longer time for high  $a_w$  samples to equilibrate to the target temperature. This most likely explains the fluctuation in the  $a_w$  readings at the beginning of data collection and the large variance in the data within a day. To minimise the effect of the sample temperature changing to reach the target temperature while collecting  $a_w$  measurements, the newer dew point hygrometer models are equipped with a user selected data collection feature where a run can be started when the sample reaches  $\pm 1$  °C or  $\pm 0.5$  °C from the target temperature.

### 5. Precision<sup>7</sup> and accuracy

The overall mean  $a_w$ , overall combined standard uncertainty and overall instrument uncertainty of all four materials for all  $a_w$  measurements is summarised in Table 12. Instrument uncertainties for all four materials were better than the precision, expressed as the standard deviation, reported by the manufacture of  $\pm 0.001$  (Water Activity Catalog, 2005) and by Richard and Labuza (1990). It is important to note that all unique  $a_w$  measurements (see Supplementary material Tables A–I) were included in the precision calculation for each material, without removing or adjusting for samples that were shown either to not be in full headspace equilibrium (i.e., corn flakes) or temperature equilibrium (i.e., such as the first six potassium chloride and jam  $a_w$  readings in Fig. 6).

Table 13 compares the  $a_w$  results of two saturated salt slurries from this study to the mean literature  $a_w$  values (Greenspan, 1976; Nyqvist, 1983; Stokes & Robinson, 1949; Young, 1967). A two-sample  $t$ -test with equal variances was used to assess the statistical difference between the two data sets. For both saturated potassium acetate slurry and saturated potassium chloride slurry, no difference was found, at  $p = 0.05$ . This result indicates that the dew point hygrometer is very accurate. Accuracy was also assessed by comparing the accuracy obtained in this study to that reported by the manufacturer,  $\pm 0.003$ . Accuracy in this study was determined as the square root of the sum of square differences between measured values and literature values across all observations divided by the degrees of freedom ( $n - 1$ ). For potassium acetate and potassium chloride, the calculated mean accuracies were 0.0002 and 0.0005, respectively.

Similar accuracy and precision results were reported for  $a_w$  values at 25 °C obtained by the dew point hygrometer for saturated salt solutions (LiCl, K<sub>2</sub>CO<sub>3</sub>, NaCl, and KCl) and water across 19 observations (Water Activity News, 2006). The mean accuracy across all solutions was reported as  $\pm 0.0006$ , while the mean precision was  $\pm 0.0005$ .

<sup>7</sup> The meaning of the term precision used here includes both repeatability (closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement) and reproducibility (closeness of the agreement between the results of successive measurements of the same measurand carried out under the changing conditions of measurement, such as the day to day measurements) of the measurement results (ISO, 1995).

**Table 13**Statistical comparison, using a two-sample  $t$ -test with equal variances, of measured  $a_w$  values of two saturated salt slurries used in this study and mean literature  $a_w$  values at 25 °C.

	This study	Literature value <sup>a</sup>
<i>Potassium acetate</i>		
Mean	0.221	0.222
Overall uncertainty	$\pm 0.0012$	$\pm 0.0042$
Number of samples ( $n$ )	60	4
$p$ -Value	0.1355	
Calculated mean accuracy	0.0002	
<i>Potassium chloride</i>		
Mean	0.845	0.843
Overall uncertainty	$\pm 0.0018$	$\pm 0.0006$
Number of samples ( $n$ )	30	4
$p$ -Value	0.0513	
Calculated mean accuracy	0.0005	

<sup>a</sup> Literature sources: Greenspan (1976), Nyqvist (1983), Stokes and Robinson (1949), and Young (1967).

### 6. Conclusions

One-way ANOVA and uncertainty analysis was carried out on hygrometer-obtained  $a_w$  measurements on four materials to determine both the significance and the magnitude of identified sources of uncertainty. The AquaLab 3TE  $a_w$  metre, termed dew point hygrometer, was used as the example hygrometer. Data analysis revealed that the significance and magnitude of the identified sources of uncertainty were dependent on material type, but that the dew point hygrometer instrument uncertainty was always significantly smaller than each of the other sources of uncertainty investigated, except for jam cup volume, which was exceeding small. Information gleaned from uncertainty analysis identified the following measurement procedures that could be used to reduce  $a_w$  measurement uncertainty for both salt and food materials: (1) in the case of glassy or inhomogeneous samples, sample protocols (e.g., cup volume, sample preparation, and sample measurement duration) need to be investigated, optimised, and standardised so as to obtain precise and accurate  $a_w$  data at the fastest measurement duration times possible; (2) depending on material type, cup volume is important to optimise so as to ensure that the water vapour pressure of the material dominates the chamber headspace; and (3) for high  $a_w$  samples, equilibrating the sample to the measurement temperature prior to  $a_w$  readings will decrease uncertainty. Overall, the dew point hygrometer studied here was found to be a precise and accurate instrument for obtaining  $a_w$  measurements for a variety of material types. Analysis of the salt and food  $a_w$  values showed that the dew point hygrometer precision for each material was better than the value reported by the manufacture ( $\pm 0.001$ ), even when all measurements were included. Comparison of the measured and literature salt  $a_w$  values showed that the dew point hygrometer accuracy was better than the  $\pm 0.003$   $a_w$  value reported by the manufacturer. Uncertainty analysis is extremely useful for identifying and reducing sources of variation associated with  $a_w$  measurements, allowing food scientists to make more informed product development, quality assurance, and product life-cycle decisions. In addition,



the uncertainty analysis used here can also be applied to investigate the uncertainty associated with other food materials and/or instruments.

### Acknowledgement

The generous support of the Cargill Women in Science Advanced Degree Scholarship is gratefully acknowledged for supported of author Xin Yu.

### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2008.12.001.

### References

- AquaLab Operator's Manual Series 3 & 3TE (Version 1.6) for "A" Models (2002). <[http://www.decagon.com/food\\_science/literature/manuals.php](http://www.decagon.com/food_science/literature/manuals.php)>. Accessed 19.06.08.
- Campbell, G., & Lewis, D., inventors (1998). Decagon Device Inc., assignee. Water activity and dew point temperature measuring apparatus and method. October 6, US patent 5,816,704.
- Chanvrier, H., Colonna, P., Valle, G., & Lourdin, D. (2004). Structure and mechanical behaviour of corn flour and starch-zein based materials in the glassy state. *Carbohydrate Polymers*, 59, 109–119.
- Christian, J. H. B., & Scott, W. J. (1953). Water relations of *Salmonellae* at 30 °C. *Australian Journal of Biological Sciences*, 6, 565–573.
- Fontana, A. J. (2005). Fundamentals of water activity seminar, sponsored by Decagon Devices Inc., Pullman, WA. In *Presented in Oakbrook, IL*. Additional information can be found at <[http://www.decagon.com/food\\_science/seminars/seminars.php](http://www.decagon.com/food_science/seminars/seminars.php)>. Accessed 20.06.08.
- Fontana, A. J. (2007). Measurement of water activity, moisture sorption isotherms, and moisture content of foods. In G. V. Barbosa-Canovas, A. J. Fontana, S. J. Schmidt, & T. P. Labuza (Eds.), *Water activity in foods: Fundamentals and applications* (pp. 155–171). Ames, IA: Blackwell Publishing.
- Franks, F. (1982). Water activity as a measure of biological viability and quality control. *Cereal Foods World*, 27(9), 403–407.
- Franks, F. (1991). Water activity: A credible measure of food safety and quality? *Trends in Food Science and Technology*, 2(3), 68–72.
- Greenspan, L. (1976). Humidity fixed points of binary saturated aqueous solution. *Journal of Research of the National Bureau of Standards – A Physics and Chemistry*, 81A(1), 89–96.
- International Organization for Standardization (ISO) (1995). *Guide to the expression of uncertainty in measurement*. Geneva: Switzerland.
- Love, D. (2004). Comparing two measuring techniques. *Food Quality Magazine*(June/July), 62–63.
- Maroto, A., Boque, E., & Ruis, F. (1999). Evaluating uncertainty in routine analysis. *Trends in Analytical Chemistry*, 18, 577–583.
- Nyqvist, H. (1983). Saturated salt solutions for maintaining specified relative humidities. *International Journal of Pharmaceutical Technology & Product Manufacture*, 4(2), 47–48.
- Richard, J., & Labuza, T. (1990). Rapid determination of the water activity of some reference solutions, culture media, and cheese using a new instrument based on the dew-point method. *Sciences des Aliments*, 10, 57–64.
- Roa, V., & Tapia de Daza, M. (1991). Evaluation of water activity measurements with a dew point electronic humidity meter. *Lebensmittel-Wissenschaft und Technologie*, 24, 208–213.
- Schmidt, S. J. (2004). *Water and solids mobility in foods. Advances in food and nutrition research* (Vol. 48). London, UK: Academic Press. pp. 1–101.
- Scott, W. (1953). Water relations of *Staphylococcus aureus* at 30 °C. *Australian Journal of Biological Science*, 6, 549–564.
- Scott, W. J. (1957). Water relations of food spoilage microorganisms. *Advances in Food Research*, 7, 83–127.
- Slade, L., & Levine, H. (1991). Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition*, 30(2–3), 115–360.
- Stokes, R. H., & Robinson, R. A. (1949). Standard solutions for humidity control at 25 °C. *Industry and Engineering Chemistry*, 21, 2013.
- van Zoonen, P., Hoogerbrugge, R., Gort, S., van de Wiel, H., & van't Klooster, H. (1999). Some practical examples of method validation in the analytical laboratory. *Trends in Analytical Chemistry*, 18, 584–593.
- Voysey, P. (1993). An evaluation of the AquaLab CX-2 system for measuring water activity. *Digest*, 124, 25–26.
- Water Activity Catalog (2005). *Water activity solutions*. <[http://www.decagon.com/food\\_science/literature/brochures.php](http://www.decagon.com/food_science/literature/brochures.php)>. Accessed 19.06.08.
- Water Activity News (2006). *Is the AquaLab still the fastest and most accurate?* Decagon devices newsletter. <[http://www.decagon.com/food\\_science/literature/newsletters.php](http://www.decagon.com/food_science/literature/newsletters.php)>. Accessed 19.06.08.
- Young, J. F. (1967). Humidity control in the laboratory using salt solution – A review. *Journal of Applied Chemistry*, 17, 241–245.
- Yu, X. (2007). *Investigation of moisture sorption properties of food materials using saturated salt solution and humidity generating techniques*. Ph.D. Dissertation. University of Illinois at Urbana-Champaign.