Dew-Point Method for the Determination of Water Activity

The single most important property of water in food systems is water activity (a_W; Taoukis et al., 1988). Throughout history the importance of controlling water in food by drying, freezing, or addition of sugar or salt was recognized for preserving and controlling food quality. Water activity is a measure of the energy status of the water in a system (or the degree to which the water is "bound"), and hence of its availability to act as a solvent and participate in chemical or biochemical reactions and growth of microorganisms. It is an important property that can be used to predict the stability and safety of food with respect to microbial growth, rates of deteriorative reactions, and chemical or physical properties (Figure A2.2.1). The water activity principle has been incorporated by various regulatory agencies (e.g., Food and Drug Administration Code of Federal Regulations Title 21) in defining safety regulations regarding growth and proliferation of undesirable microorganisms, potentially hazardous foods, standards of several preserved foods, and packaging requirements (Fontana, 1998).

The Basic Protocol describes the determination of water activity of a product using a chilled mirror dew-point water activity meter. Dew point is a primary measurement of vapor pressure that has been in use for decades (Harris, 1995). Dew-point instruments are accurate, fast, simple to use, and precise (Richard and Labuza, 1990; Snavely et al., 1990; Roa and Tapia de Daza, 1991). In a dew-point instrument, water activity is measured by equilibrating the liquid-phase water in the food sample with the vapor-phase water in the headspace, and then measuring the vapor pressure of the headspace. The basic principle involved in dew-point determinations of vapor pressure in air is that air may be cooled without change in water content until it saturates. The dew-point temperature is the temperature at which the air reaches saturation. It is determined in practice by measuring

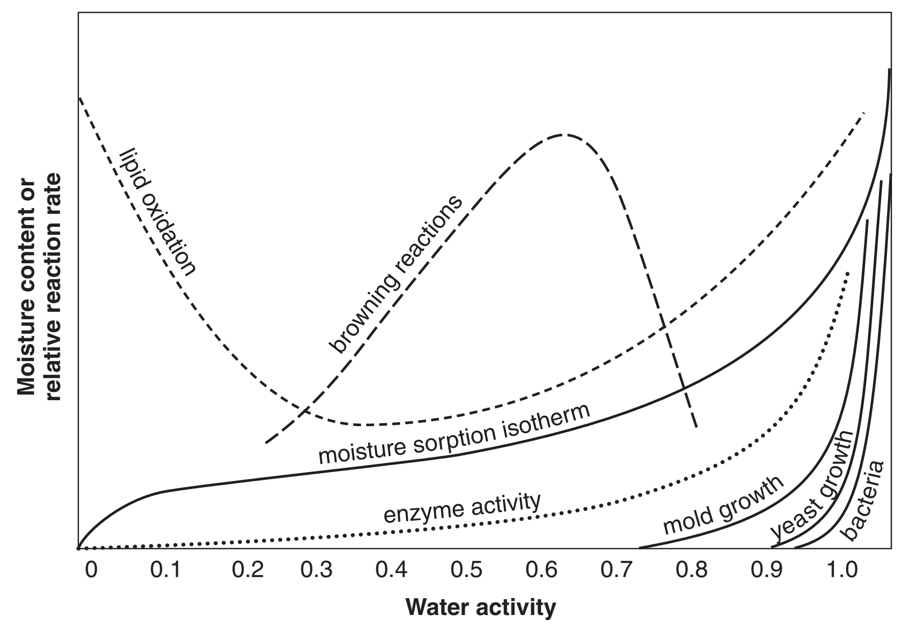

Figure A2.2.1 Water activity stability map (adapted form Labuza, 1970). A representation of a typical sorption isotherm for food materials and of the effects of water activity on the relative reaction rates of several chemical processes, as well as the growth of microorganisms, in foods are shown.

Contributed by Anthony J. Fontana

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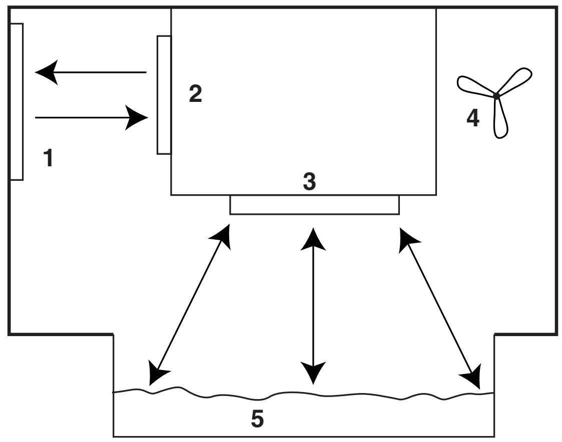

Figure A2.2.2 A representation of a modern dew-point chamber consisting of (1) mirror, (2) optical sensor, (3) infrared thermometer, (4) internal chamber fan, and (5) sample cup with sample.

the temperature of a chilled mirror when condensation starts. The water activity of the sample is the ratio of the saturation vapor pressure at dew-point temperature to the saturation vapor pressure at the sample temperature.

In a modern dew-point instrument, a sample is equilibrated within the headspace of a sealed chamber containing a mirror, an optical sensor, an internal fan, and an infrared thermometer (Figure A2.2.2). At equilibrium, the relative humidity of the air in the chamber is the same as the water activity of the sample. A thermoelectric (Peltier) cooler precisely controls the mirror temperature. An optical reflectance sensor detects the exact point at which condensation first appears; a beam of infrared light is directed onto the mirror and reflected back to a photodetector, which detects the change in reflectance when condensation occurs on the mirror. A thermocouple attached to the mirror accurately measures the dew-point temperature. The internal fan is for air circulation to reduce vapor equilibrium time and to control the boundary layer conductance of the mirror surface (Campbell and Lewis, 1998). Additionally, an infrared thermometer measures the sample surface temperature. Both the dew-point and sample temperatures are then used to determine the water activity. The range of a commercially available dew-point meter is 0.030 to 1.000 a_w, with a resolution of ±0.001 a_w and accuracy of ±0.003 a_w. Measurement time is typically less than 5 min. The performance of the instrument should be routinely verified as described in the Support Protocol.

BASIC PROTOCOL

USING A DEW-POINT INSTRUMENT

Dew-point measurement is a primary method based on fundamental thermodynamics principles and as such does not require calibration. However, the instrument performance needs to be verified using salt standards and distilled water before sampling (see Support Protocol). To obtain accurate and reproducible water activity results with a dew-point instrument, temperature, sensor cleanliness, and sample preparation must be considered. Equipment should be used and maintained in accordance with the manufacturer's instruction manual and with good laboratory practice. If there are any concerns, the manufacturer of the instrument should be consulted. Guidelines common to dew-point instruments for proper water activity determinations are described in this protocol. The manufacturer's instructions should be referred to for specifics.

The type (e.g., liquid, solid, powder, gel, syrup, emulsion, granule) and range of food samples (raw ingredients to final products) for water activity measurement are immense. The amount of sample required for measurement is typically 5 to 10 ml. A homogeneous and representative sample should be prepared and placed into the sample cup. For the majority of samples, no preparation is necessary; the sample is simply placed into the cup. Multicomponent (e.g., muffin with raisins or pizza) and coated samples (e.g., breaded foods or chocolate-covered bar) may have to be sliced, crushed, or ground in order to obtain a representative sample. If sample preparation is necessary, then a consistent technique must be used with each sample to ensure reproducible results.

Materials

Sample
Dew-point water activity instrument (e.g., AquaLab Series 3, Decagon Devices)
Disposable sample cups (and optional lids; available from the instrument manufacturer)

1. Place a dew-point water activity instrument on a level surface and in a relatively temperature-stable environment. Allow instrument to warm up for ≥15 min after turning on for optimal performance.

   Performance verification should be conducted before sampling at least once per day, at each shift, or if readings become unstable (see Support Protocol).

2. Prepare a sample (e.g., liquid, solid, powder, gel, syrup, emulsion, granule) that is homogeneous and representative of the entire product.

   For detailed instructions on samples requiring special handling, see Critical Parameters and Troubleshooting, discussion of sample preparation.

3. Place sample (5 to 10 ml) in a disposable sample cup, completely covering the bottom of the cup, if possible. Place sample into sample cup in a timely manner to minimize moisture loss or gain during handling. If sample is not to be tested immediately, place a sample cup lid on the cup to prevent moisture transfer with the room atmosphere.

   Do not fill the sample cup more than half full. Overfilled cups will contaminate the sensors in the sampling chamber.

4. Wipe the cup rim with a lint-free tissue (e.g., Kimwipe), making sure that the rim and outside of the sample cup are clean and free of any sample material.

5. Place sample cup into the instrument. Check top lip of the cup to make sure no sample is protruding above its rim.

   Remember, an overfilled sample cup will contaminate the chamber's sensors.

6. Seal the measurement chamber, being especially careful not to splash or spill the sample and contaminate the chamber.

7. Start water activity measurement.

   This will start the read cycle, in which the dew-point temperature is repeatedly measured until vapor equilibrium is reached. The dew-point instrument will signal the operator when vapor equilibrium is achieved, and will display the final water activity and sample temperature on its screen.

   Length of read times may vary depending on temperature differences between the chamber and the sample, and on the properties of the sample.

8. Remove sample from instrument after measuring water activity.

   Never leave samples inside the chamber overnight or for extended periods of time, as this can contribute to contamination of the chamber.

SUPPORT PROTOCOL

PERFORMANCE VERIFICATION FOR DEW-POINT WATER ACTIVITY INSTRUMENT

Performance verification should be conducted before sampling at least once per day, at each shift, or if readings become unstable. Performance of the instrument is verified using salt standards that have known water activities. Either saturated or unsaturated salt solutions are suitable for verifying instrument performance. The choice of using saturated or unsaturated salt solutions is dependent upon the water activity range of the samples, temperature variability, cost, and ease of use. Saturated salt slurries have specific water activity values depending upon the chosen salt and the temperature. Some salts are hazardous and may not be appropriate in a food-testing laboratory. On the other hand, unsaturated salt solutions can be easily made to any water activity value desired. Unsaturated salt solutions (sodium chloride and lithium chloride) are much less temperature dependent than saturated salt slurries, and are thus stable over a wide temperature range. Instrument manufacturers provide certified unsaturated salt solutions at various water activity levels in individual prepackaged vials.

Additional Materials *(also see Basic Protocol)*

Saturated or unsaturated salt standards (see recipes)

Set up instrument

1. Place a dew-point water activity instrument on a level surface and in a relatively temperature-stable environment. Allow instrument to warm up for ≥15 min after turning on for optimal performance.

2. Choose a salt standard with a water activity level that is slightly below the water activity of sample to be measured. Make sure that salt standard is at ambient temperature before loading it into the instrument.

3. Fill a sample cup half full (~7 ml) with selected salt standard. Place sample into sample cup in a timely manner to minimize moisture loss or gain during handling.

   Do not fill the sample cup more than half full. Overfilled cups will contaminate the sensors in the sampling chamber.

4. Wipe the cup rim with a lint-free tissue, making sure that the rim and outside of the sample cup are clean and free of any sample material.

5. Place sample cup into the instrument. Check top lip of the cup to make sure no sample is protruding above its rim.

6. Seal the measurement chamber, being careful not to splash or spill the solution and contaminate the chamber.

Measure performance

7. Start the water activity measurement (see Basic Protocol, steps 7 and 8). Make two measurements.

   The water activity readings should be within ±0.003 a_w of the given value of the salt standard. If they are, proceed to step 10. If they are not, proceed to step 8.

8. If the readings are consistently outside the stated water activity values by more than ±0.003 a_w, the sensor chamber is contaminated. Clean chamber according to manufacturer's instructions.

9. Repeat step 7. If after cleaning, the readings are still outside of the stated water activity value by more than ±0.003 a_w, a calibration offset has probably occurred. Adjust the reading of a salt solution to its correct value according to manufacturer's instructions, then repeat step 7 again.

10. Prepare a sample cup half full of distilled water and make two readings.

    The second water activity reading should be 1.000 ± 0.003 a_w. If it is, the instrument is ready for use (see Basic Protocol). If it is not, proceed to step 11.

11. If the salt reading is correct and distilled water is not, it is probably due to slight contamination of the sensor chamber. Clean chamber according to manufacturer's instructions, then repeat step 7.

REAGENTS AND SOLUTIONS

*Use deionized or distilled water in all recipes and protocol steps. For common stock solutions, see **APPENDIX 2A**; for suppliers, see **SUPPLIERS APPENDIX**.*

Saturated salt standards

Start with a salt and add water in small increments, stirring well with a spatula after each addition. Continue until the salt can absorb no more water, as evidenced by free liquid (where it will take on the shape of the container but will not easily pour). Store indefinitely at room temperature in a manner to prevent substantial moisture gain or evaporation losses.

When a salt standard is prepared so that it consists mostly of liquid with a few crystals in the bottom, it can result in a layer of less-than-saturated solution at the surface, which will produce a higher reading than anticipated. Conversely, solid crystals protruding above the surface of the liquid can lower the readings.

Table A2.2.1 lists the water activity of various salt slurries at 20° and 25°C (Greenspan, 1977). Greenspan provides additional information on these and other salts over a wider temperature range.

Saturated salt solutions in the form of salt slurries can be prepared by several other methods (see Stoloff, 1978; Mulvaney, 1995).

Unsaturated salt standards

Prepare salt solution at desired concentration (see Table A2.2.2 for NaCl or LiCl). Store indefinitely at room temperature in a manner to prevent substantial moisture gain or evaporation losses.

Unsaturated salt solutions of different concentrations make excellent standards. These solutions are easily made and are relatively unaffected by temperature over a wide range of concentrations (Chirife and Resnik, 1984). Table A2.2.2 lists the water activity of various sodium chloride and lithium chloride solutions according to Robinson and Stokes (1965). The data in Robinson and Stokes can be used to compute the a_w for other unsaturated salt solutions.

Dew-point instrument manufacturers also provide certified unsaturated salt solutions at various water activity levels in individual prepackaged vials.

Table A2.2.1 Water Activity of Selected Saturated Salt Standards[a]

Salt	a_w at 20°C	a_w at 25°C
LiCl	0.113 ± 0.003	0.113 ± 0.003
MgCl	0.331 ± 0.002	0.328 ± 0.002
K_2CO_3	0.432 ± 0.003	0.432 ± 0.004
$Mg(NO_3)_2$	0.544 ± 0.002	0.529 ± 0.002
NaCl	0.755 ± 0.001	0.753 ± 0.001
KCl	0.851 ± 0.003	0.843 ± 0.003
K_2SO_4	0.976 ± 0.005	0.973 ± 0.005

[a] Adapted from Greenspan (1977). Numbers rounded to nearest thousandth.

Table A2.2.2 Water Activity of Unsaturated Sodium Chloride and Lithium Chloride Solutions at 25°C[a]

Sodium chloride		Lithium chloride	
Molality	a_w	Molality	a_w
0.1	0.996	0.1	0.997
0.2	0.993	0.5	0.983
0.3	0.990	1.0	0.964
0.4	0.986	1.4	0.948
0.5	0.983	2.0	0.921
0.6	0.980	2.5	0.897
0.7	0.976	3.0	0.870
0.8	0.973	3.5	0.842
0.9	0.970	4.0	0.812
1.0	0.966	4.5	0.780
1.2	0.960	5.0	0.747
1.4	0.953	5.5	0.713
1.6	0.946	6.0	0.679
1.8	0.938	7.0	0.609
2.0	0.931	8.0	0.539
2.2	0.924	9.0	0.473
2.4	0.916	10.0	0.412
2.6	0.908	11.0	0.356
2.8	0.901	12.0	0.307
3.0	0.893	13.0	0.266
3.2	0.885	14.0	0.230
3.4	0.876	15.0	0.200
3.6	0.868	16.0	0.175
3.8	0.860	17.0	0.155
4.0	0.851	18.0	0.138
5.0	0.807	19.0	0.123
6.0	0.760	20.0	0.110

[a] Adapted from Robinson and Stokes (1965).

COMMENTARY

Background Information

Water activity (a_w) is an important property for food safety. It predicts food safety and stability with respect to microbial growth, chemical or biochemical reaction rates, and physical properties (Figure A2.2.1). By measuring the water activity of foodstuffs, it is possible to predict which microorganisms will be potential sources of spoilage and infection. Controlling water activity is also an important way to maintain the chemical stability of foods. Water may influence chemical reactivity in different ways. It may act as a solvent or reactant, or change the mobility of the reactants by affecting the viscosity of the food system (Leung, 1987). Water activity influences nonenzymatic browning, lipid oxidation, degradation of vitamins, enzymatic reactions, protein denaturation, starch gelatinization, and starch retrogradation. Finally, a_w plays a significant role in the physical properties of foods, such as texture and shelf life. Water activity is the driving force for moisture migration within a food sample.

Reliable laboratory instrumentation is required to guarantee the safety of food products and enforce government regulations. Two main types of commercial instruments are available. One uses chilled mirror dew-point technology, while the other measures relative humidity with sensors that detect changes in electrical resistance or capacitance. Each has advantages and disadvantages. The methods vary in accuracy, repeatability, speed of measurement, stability in calibration, linearity, and convenience of use.

Water activity is derived from fundamental principles of thermodynamics and physical chemistry. It is defined from the equilibrium state:

$\mu = \mu_0 + (RT \times \ln[f/f_o])$

where μ is the chemical potential of the system, i.e., thermodynamic activity or energy per mole of substance; μ_0 is the chemical potential of the pure material at the temperature T; R is the gas constant; f is the fugacity or the escaping tendency of a substance; and f_0 is the escaping tendency of pure material (van den Berg and Bruin, 1981). The activity of a species is defined as $a = f/f_0$. When dealing with water, a subscript is designated for the substance, $a_w = f/f_0$, where a_w is activity of water, or the escaping tendency of water in a system divided by the escaping tendency of pure substance with no radius of curvature. For practical purposes, under most conditions in which foods are found, the fugacity is closely approximated by the vapor pressure ($f \approx p$):

$a_w = f/f_0 \cong p/p_0$

Equilibrium is obtained in a system when μ is the same everywhere in the system. Equilibrium between the liquid and the vapor phases implies that μ is the same in both phases. It is this fact that allows the measurement of the vapor phase to determine the water activity of the sample.

The water activity of a food describes the energy status of water in a food and, hence, its availability to act as a solvent and participate in chemical or biochemical reactions (Labuza, 1977). Figure A2.2.1 is a global stability map of foods, showing stability as a function of a_w (Labuza, 1970). Water's ability to act as a solvent, medium, and reactant increases with increasing water activity (Labuza, 1975).

Critical Parameters and Troubleshooting

To obtain accurate and reproducible water activity results with a dew-point instrument, the temperature, sensor cleanliness, and sample preparation must be considered. Temperature influences water activity measurements in two ways; one is its effect on the water activity in the sample and the second is the effect on the dew-point measurement.

Temperature effect on sample water activity

Water activity is temperature dependent. Temperature changes water activity due to changes in water binding, dissociation of water, solubility of solutes in water, or the state of the matrix. Although solubility of solutes can be a controlling factor, control is usually from the state of the matrix. Since the state of the matrix (glassy versus rubbery) is dependent on temperature, one should not be surprised that temperature affects the water activity of the food. The effect of temperature on the water activity of a food is product specific. Some products increase a_w with temperature, others decrease a_w with increasing temperature, while most high-moisture foods have negligible change with temperature. Therefore, one cannot predict even the direction of the change of water activity with temperature, since it depends on how temperature affects the factors that control water activity in the food.

The importance of temperature control is significant for laboratory comparisons, accelerated shelf-life studies, and packaging requirements. Also, temperature may be essential when measuring a_w levels near critical values, especially for government regulations or critical control points. The precision required in water activity applications will determine the need for temperature control. Several reasons for sample temperature control are:

Research purposes. These include studies that examine the effects of temperature on the a_w of a sample or follow accelerated shelf life conditions, as well as those that compare the a_w of different samples independent of temperature. There are many shelf life, packaging, and isotherm studies in which the added feature of temperature control would be very beneficial.

To comply with government or internal regulations for specific products. Though the a_w of most products varies by less than $\pm 0.002\ a_w$ per °C, some regulations require measurement at a specific temperature. The most common specification is 25°C, though 20°C is sometimes indicated.

To minimize extreme ambient temperature fluctuations. If the laboratory and dew-point instrument temperatures fluctuate by as much as ±5°C daily, water activity readings may vary by $\pm 0.01 a_w$. Often, this much uncertainty in sample a_w is unacceptable, so there is a need for a temperature-controlled model.

Chilled mirror dew-point instruments are available in a variety of temperature-controlled models from the manufacturer.

Temperature effect on dew-point measurement

Temperature must be defined for water activity measurements. Changes in sample temperature will cause the water activity readings to change until the sample temperature stabilizes. When the sample temperature stabilizes within one or two degrees of the chamber temperature, then accurate and rapid measurements are made. Samples that are 4°C colder or warmer than the instrument will need to equilibrate to the instrument temperature before accurate readings can be made. If a sample is frozen or refrigerated before testing, it needs to be brought back to ambient temperature so that condensation moisture can be reabsorbed into the product. Samples with high water activity that are above the chamber temperature can cause condensation within the chamber, which will adversely affect subsequent readings. Some instruments display a warning message if the sample temperature is above the chamber temperature.

Sensor performance

The accuracy of a dew-point instrument is vitally dependent on keeping the instrument clean. Commercial dew-point instruments will provide accurate water activity measurements as long as their internal sensors are not contaminated by improperly prepared samples. If the sensors are contaminated, they must be cleaned. Careful preparation and loading of samples will lengthen the time between cleanings. The rim and outside of the sample cup should be kept clean. Any excess sample material should be wiped from the rim of the cup with a clean lint-free tissue. The rim of the cup must be able to form a vapor seal with the sensor block when the water activity measurement is started. Any sample material left on the cup rim will be transferred to the chamber block, preventing this seal and contaminating future samples. The sample cup should not be filled more than half full, as overfilled cups are likely to result in spilled sample and contamination of the sensors in the chamber. When measuring liquid samples, it is important to be gentle when inserting the sample so as not to spill the solution out of the dish. If the drawer is pushed or pulled too quickly, the solution will spill and contaminate the chamber. Sensor performance and cleanliness is verified by using salt standards and distilled water (see Support Protocol).

Sample preparation

For most foods, water activity is an important property of stability and safety. Thus, samples ranging from raw ingredients to finished goods should be analyzed for water activity. The sample to be tested must be representative of the entire product. Most samples do not need any further preparation; they can simply be placed in the cup and measured for water activity. Some precautions should be taken for multicomponent and coated samples to ensure that a representative and homogeneous sample is tested.

For multicomponent and coated samples there are two methods of preparation, depending on the purpose of the water activity analysis. The first method is to obtain a representative sample of the entire product by crushing or grinding the sample. If the sample is to be crushed or ground, then a consistent technique is necessary to ensure reproducible results. For example, if one sample is ground for 15 sec and a second sample is ground for 1 min, then the second sample will have smaller particles with more surface area and a greater chance of gaining or losing moisture to the environment. In addition, the time between grinding and the water activity measurement should be consistent, or additional precautions should be taken to prevent moisture exchange with the room air.

The second method is to separate the components and measure each individually. Since water activity is a driving force for moisture migration, it might be necessary to know the water activity values for the individual components. For example, in a flake cereal with fruit pieces, it might be important to know the individual component water activities. The water activity of the cereal flakes and fruit pieces should be as close as possible to prevent moisture migration, which would cause the flakes to become soggy and the fruit pieces to become hard and brittle.

In addition to equilibrium between the liquid-phase water in the sample and the vapor phase, the internal moisture equilibrium of the sample is important. If a system is not at internal moisture equilibrium, one might measure a steady vapor pressure (over the period of measurement) that is not the true water activity of the system. An example of this might be a baked good or a multicomponent food. Initially out of the oven, a baked good is not at internal equilibrium; the outer surface is at a lower water activity than the center of the baked good. One must wait a period of time in order for the water to migrate and the system to come to internal equilibrium. It is therefore important to remember the restriction of the definition of water activity to equilibrium.

Limitations

Chilled mirror dew-point instruments have limitations with certain volatile compounds that co-condense on the mirror surface with water vapor. Samples with high levels of propylene glycol require special sampling procedures to obtain accurate readings. Samples that contain propylene glycol in concentrations >10% will not damage the instrument, but a_w values for consecutive samples will not be accurate. Propylene glycol condenses on the mirror during the reading process, but does not evaporate from the mirror as water does. As a result, the very first reading will be somewhat accurate, but subsequent readings will not be accurate unless propylene glycol is cleared from the chamber after each measurement. Running a sample consisting of activated charcoal after each sample containing propylene glycol accomplishes this. Dew-point instruments will give accurate readings on most samples containing alcohols, except those containing ethanol. Samples containing ethanol will yield elevated water activity results.

Anticipated Results

Commercial water activity instruments that use the chilled mirror dew-point method described above measure water activity values between 0.030 and 1.000 a_w. They attain a precision of 0.001 a_w with an accuracy of ± 0.003 a_w and a resolution of ± 0.001 a_w.

Time Considerations

Measuring both the dew point and sample temperature eliminates the need for complete thermal equilibrium and reduces measurement times to <5 min for most samples. Because water activity is an equilibrium measurement, multicomponent samples, samples that have outside coatings, or high-fat samples may take longer to equilibrate. In addition, some extremely dry samples, dehydrated samples, highly viscous samples, or glassy compositions may have increased read times due to their moisture sorption characteristics. Samples like these may take >5 min to give an accurate reading or may require multiple readings. To reduce the time needed to take an a_w reading for coated, dried, or glassy samples, one can crush, slice, or grind the sample before placing it in the sample cup. This increases the surface area of the sample, thus increasing the equilibration rate. When samples are crushed, ground, or sliced, the method should be consistent in order to ensure reproducible results.

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Internet Resources

www.decagon.com/aqualab/

Web site containing dew-point method and theory information.

Contributed by Anthony J. Fontana
Decagon Devices
Pullman, Washington