Water Activity (A_w) Analysis

Purpose: To determine the amount of available active water in any raw material, spice or herb (see Notes 1 & 2).

A. Apparatus

Water activity instruments are available from multiple vendors. The key elements of a water activity measurement system are a temperature-controlled chamber (usually to keep the sample and vapor space at 25ºC ± 1ºC) and a hygrometer (humidity) sensor. Two primary types of sensors are used.

1. Capacitance hygrometer - the capacitance of a dielectric membrane varies as a function of moisture and is a selective measure of vapor phase water.
2. Dew point hygrometer – an optical sensor is used to measure the temperature at which condensation forms on a chilled mirror.

Each type of sensor may have application limitations because of interferences from other volatile components in the sample. For example, capacitance hygrometers are affected by CH_3COOH (acetic acid). Dew point hygrometers can respond to condensables with lower critical temperatures than H_2O and might not be appropriate for samples that contain significant amounts of non-aqueous volatile components such as alcohols or essential oils.

B. Reagents

Consult instrument manufacturer guidelines for appropriate standards and reagents and their purity requirements. Standard salt solutions or slushes for reference measurements are described in various publications (see Procedures and References sections). High purity water (e.g. HPLC grade) will provide $A_w = 1.000$.

C. Preparation of Sample

Consult manufacturer guidelines for preparation. The sample should be at or near 25ºC before testing. Samples with multiple components or samples that have wide particle size range (such as crushed chilies, etc…) also can be evaluated but will take a longer time to equilibrate and should be analyzed multiple times and averaged for an accurate estimate of the A_w. Very large sample pieces can be cut for easier placement in the measurement cup. Some manufacturers offer large sample cups to allow for testing of a more representative portion from samples with poor uniformity.

D. Procedure

1. Follow instrument documentation for proper testing procedures. Best practices for sample and instrument management include the following.

a. Keep desiccant in the chamber to keep the sensor dry when the meter is not in use. It is also helpful to load the chamber with dessicant after running high A_w samples.

b. It is important not to overfill sample cups. Overfilled sample cells or dust on the rim of the sample cell can lead to contaminated or corroded sensors and are the most common cause of erroneous results. The sensor and chamber must then be cleaned or repaired. Fill cup according to manufacturer instructions.

c. Testing must be conducted in a stable environment (i.e. a room with excellent temperature and humidity control). Results obtained in high-moisture surroundings may be over estimated by 0.100 units or more. (Note 3)

2. Instrument performance should be verified on the day of use by measuring at least two standards bracketing the expected results of your samples. The A_w values obtained for each standard should fall within 0.003 units of the reference value. Certified standards can be purchased from instrument manufacturers. Standards can also be prepared as "slushes" of reagent grade substances by partially filling the sample cup with the substance and adding and mixing water in 1-2 mL increments until free liquid is first observed. Some common standard slushes and their A_w values at 25ºC are: $MgCl_2$ (0.328), NaBr (0.576), NaCl (0.753), KBr (0.809), KCl (0.843) and K_2SO_4 (0.973). Slushes are mixtures of solid salt and their saturated aqueous solutions. Their preparation is described in AOAC method 978.18.

3. If the initial standard solution readings are not correct, clean the sensor/chamber and repeat the measurements. If the standard solution values remain incorrect, then follow manufacturer instructions to adjust the instrument or arrange for repair.

E. Calculations

Instrument response is typically standardized against reference solutions. See instrument documentation for details. No calculations are needed unless the instrument reports values as percent relative humidity (RH%). In this case, A_w is RH%/100.

F. Statistics

Results of the ASTA Check Sample Program between Sep 2008 – May 2010 (seven rounds, approximately 20 labs per round) show lab-to-lab reproducibility for this method of ± 3% relative standard deviation for dehydrated capsicum and ± 5% relative standard deviation for dehydrated black pepper.

Interlaboratory testing between three ASTA member labs produced inflated A_w readings by dew point sensor in a sample of clove with high volatile oil (VO) content (see Table below). The differences were relatively small in this study but laboratories should not assume that this will always be the case when using dew point hygrometers. Laboratories that employ dew point hygrometers for high VO materials should consider if detector bias could affect determination of the safety and stability of a material. In cases where it might, the laboratory

should establish the degree of bias for each sample type by comparing results from a more selective humidity sensor.

	Ground Cassia (VO 5.2%)	Ground Clove (VO 24%)	Equipment used
Lab 1	0.647	0.724	Dew point hygrometer
Lab 2	0.637-0.640	0.727-0.724	Dew point hygrometer
Lab 2	0.639-0.641	0.716-0.719	Capacitance hygrometer
Lab 3	0.650	0.704	Capacitance hygrometer

G. Notes

1. Water activity (A_w) is expressed as the ratio of the vapor pressure of water above a sample to the same vapor pressure of pure water, at the same temperature. A_w is expressed in a 0.000-1.000 range with 0 being absolutely no active water available and 1 being 100% active water. Water activity measurement is temperature sensitive and is typically performed at 25°C.

2. The higher the A_w value of a product, the more likely that such product could support the growth of microorganisms, including pathogenic organisms, by providing the available water needed for their growth. The determination of A_w will help in ensuring the quality and integrity of supplies and their safety. The following chart shows the type of microbiological growth that could occur at various levels of A_w.

A_w	**Microorganisms inhibited by lowest A_w in this range**
0.95-1.00	Growth inhibited for Pseudomonas, Escherichia, Proteus, Shigella, Klebsiella, Bacillus, Clostridium perfringens, some yeasts.
0.91-0.94	Growth inhibited for Salmonella, Vibrio parahaemolyticus, Clostridium botulinum, Serratia, Lactobacillus, Pediococcus, some molds, yeasts (Rhodotorula, Pichia). Toxin production inhibited for all bacteria except Staphylococcus aureus.
0.87-0.91	Growth inhibited for many yeasts (Candida, Torulopsis, Hansenula), Micrococcus.
0.80-0.87	Growth inhibited for most molds (mycotoxigenic penicillia), Staphylococcus aureus, most Saccharomyces, Debaryomyces (yeast). Toxin production inhibited for Staphylococcus aureus.
0.75-0.80	Growth inhibited for most halophilic bacteria, mycotoxigenic aspergilli. Toxin production inhibited for molds.
0.65-0.75	Growth inhibited for xerophilic molds (Aspergillus chevalieri, A. candidus, Wallemia sebi), Saccharomyces bisporus.
0.61-0.65	Osmophilic yeasts (Zygosaccharomyces rouxii), few molds (Aspergillus echinulatus, Monascus bisporus).
< 0.61	No microbial proliferation or production.

Adapted from (a) Barbosa-Cánovas, G.V.; Fontana, A,J.; Schmidt, S.J.; Labuza, T.P. (ed.) "*Water Activity in Foods,"* Blackwell Publishing (Ames, IA), 2007 and (b) Beuchat, L.R. "Water Activity and Microbial Stability," *Fundamentals of Water Activity,* IFT Continuing Education Committee (Anaheim, CA), 2002.

3. Adequate environmental control is critical. AOAC Official Method 978.18 specifies that test systems be placed within a forced draft cabinet with internal volume of greater than 0.06 m^3 and temperature control of 25 ± 1ºC. Performance of the method and instrument under environmental conditions that are controlled by other means must be supported by appropriate method validation results.

H. References

AOAC Official Method 978.18, "Water activity of canned vegetables."

I. Revision history

October, 2010, original version