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APPLICATION NOTE

WATER ACTIVITY
IN CANNABIS



With the legalization of cannabis-based products for medicinal and, in some regions, recreational use, the need to implement safety initiatives has emerged. Microbial contamination in either dried buds, extracted oils, or processed edibles can result in allergic reactions, respiratory complications, or foodborne illnesses. In addition, breakdown due to chemical reactions can result in changes in efficacy and potency. Water activity is an effective tool used in the food and pharmaceutical industries to maximize microbial, chemical, and physical stability. It provides this same safety and control to the cannabis market and it is important that cultivators and processors understand water activity and how to maximize its usefulness. Safety regulations for the cultivation and processing of cannabis-based products is currently handled at the state level, resulting in inconsistent recommendations. As a result, not all states currently require water activity testing of cannabis. However, based on its established relationship with common safety and quality modes of failure, it should be the most important analytical test run by anyone in the cannabis market.

THEORY OF WATER ACTIVITY

Water activity is defined as the energy status of water in a system and is rooted in the fundamental laws of thermodynamics through Gibb's free energy equation. It represents the relative chemical potential energy of water as dictated by the surface, colligative, and capillary interactions in a matrix. Practically, it is measured as the partial vapor pressure of water in a headspace that is at equilibrium with the sample, divided by the saturated vapor pressure of water at the same temperature. The water activity covers a range of 0 for bone dry conditions up to a water activity 1.00 for pure water, resulting from the partial pressure and the saturated pressure being equal. Water activity is often referred to as the 'free water' and while useful when referring to higher energy, it is incorrect since 'free' is not scientifically defined and is interpreted differently depending on the context. As a result, the concept of free water can cause confusion between the physical binding of water, a quantitative measurement, and the chemical binding of water to lower energy, a qualitative measurement. Rather than a water activity of 0.50 indicating 50% free water, it more correctly indicates that the water in the product has 50% of the energy that pure water would have in the same situation. The lower the water activity, the less the water in the system behaves like pure water. Water activity is measured by equilibrating the liquid phase water in the sample with the vapor phase water in the headspace of a closed chamber and measuring the Equilibrium Relative Humidity (ERH) in the headspace using a sensor. The relative humidity can be determined using a resistive electrolytic sensor, a chilled mirror sensor, or a capacitive hygroscopic polymer sensor. Instruments from Novasina, like the LabMaster NEO, utilize an electrolytic sensor to determine the ERH inside a sealed chamber containing the sample. Changes in ERH are tracked by changes in the electrical resistance of the electrolyte sensor. The advantage of this approach is that it is very stable and resistant to inaccurate readings due to contamination by Cannabis when a combination filter is used, a particular weakness of the chilled mirror sensor. The resistive electrolytic sensor can achieve the highest level of accuracy and precision with no maintenance and infrequent calibration. While water activity is an intensive property that provides the energy of the water in a system, moisture content is an extensive property that determines the amount of moisture in a product. Water activity and moisture content, while related, are not the same measurement. Moisture content is typically determined through loss-on-drying as the difference in weight between a wet and dried sample. While useful as a measurement of purity and a standard of identity, as this paper will describe, moisture content does not correlate as well as water activity with microbial growth, chemical stability, or physical stability. Water activity and moisture content are related through the moisture sorption isotherm.

WATER ACTIVITY MEASUREMENT OF CANNABIS

The measurement of the water activity of Cannabis dried flower and other Cannabis containing products can pose challenges due to the high level of volatiles including terpenes and cannabinoids. These volatiles can be absorbed by or interfere with the sensor being used to measure water activity. If the sensor is not protected through some type of intervention, there is the potential for degradation of the sensor or incorrect testing results. The resistive electrolytic sensor can accurately measure water activity in the presence of Cannabis related volatiles, but to prevent it from degrading over time, the sensor should be protected with a filter. The filter recommendation from Novasina when measuring Cannabis is the eVC-21/eVALC combo for the Lab-Touch and LabSwift and the eVC-18/ eVALC combo filter for the Labmaster NEO. A recent testing study was conducted by Novasina using a LabMaster NEO where the electrolytic sensor was exposed to Cannabis flower without any protective filters for varying time periods to give more insight into the impact of Cannabis volatiles. The results of this study are shown in Table 1. The study indicated that exposure to Cannabis without a protective filter did result in changes in water activity readings on the Sal-T standards. However, the degree of change was not the same for all of the standards. The change due to exposure increased with the water activity of the standard with the 0.576 aw standard showing a maximum change of 0.003 aw, the 0.753 aw standard a maximum change of 0.005 aw, and the 0.8434 aw standard a maximum change of 0.02 aw. This would indicate that higher water activity readings are more impacted by exposure of the sensor to Cannabis than readings below 0.753. As these results were all collected without a filter, they represent a worst-case scenario, and the effects would be reduced and delayed when the correct protective filter is used. The results in Table 1 also indicate that the volatile effect on the electrolytic sensor from Cannabis can be reversed through prolonged exposure to the regeneration kit.

Testing Condition	0.8434 aw	0.7530 aw	0.5760 aw
Initial Reading	0.8395	0.7499	0.5776
After 1 Hour Exposure to Cannabis at 25C	0.8446	0.7516	0.5783
After 2.5 Hours Exposure to Cannabis at 25C	0.8456	0.7529	0.5779
After 20 Hours Exposure to Cannabis at 25C	0.8637	0.7582	0.5777
After 30 min exposure to Regeneration Kit at 50C	0.8290	0.7506	0.5754
After 3 day exposure to Regeneration Kit at 25C	0.8452	0.7537	0.5792

Table 1. Water activity testing results with 3 salt standards after varying exposures to Cannabis with no protective filter and Novasina's regeneration kit.

The results of this study combined with feedback received from customers conducting high frequency testing on Cannabis have made it possible to provide updated best practices for testing Cannabis. The current recommendations from Novasina when testing Cannabis are:

1. Utilize the recommended filter for the instrument being used for testing (eVC-21/eVALC combo for the LabTouch and LabSwift and the eVC-18/ eVALC combo filter for the Labmaster NEO)
2. Change the filter about every 1-2 months.
3. Obtain a regeneration kit from Novasina and utilize it frequently.
 - a. Some users are currently using the kit each evening, in between shifts, and not seeing any problems even under extreme testing frequencies.
 - b. If testing frequency is less than 5 per day, use of the regeneration kit can be limited to only when the verification of salt standards moves outside the tolerance limits.
4. Do not leave Cannabis samples in the instrument for long periods of time when testing is not being conducted.

WATER ACTIVITY AND US STATE REGULATIONS

Currently, governmental oversight of the production and selling of cannabis is handled on the state level with the actual state department varying by state. Consequently, regulations vary extensively between states. Water activity currently only appears in the regulations/guidance documents for a few states and these tend to be the states that have had some form of legalized cannabis for the longest time and therefore have the most mature regulations. It is expected that additional states will add water activity testing requirements to their regulations as markets mature; and if regulations move to the federal level, water activity will be included in any national regulations/guidelines.

States that currently include water activity in their cannabis regulations include:

1) NEVADA

NAC 453A.654 Required quality assurance tests;
submission of wet marijuana for testing. NAC 453A.550
“Potentially hazardous marijuana products and ingredients“ defined.

2) CALIFORNIA

CA Section 19344 - 5322 Water activity and moisture content

3) OREGON

OAR 333-007-0300 to 333-007-0500 and OAR 333-064-0100 to 333-064-0130
Must test water activity on Usable marijuana for sales or for further processing

4) WASHINGTON

WAC 314-55-102 Quality assurance testing Part (i) Moisture analysis. Must test water activity on usable marijuana intended for retail sale or for further processing

In addition to the inclusion of water activity in state cannabis regulations, ASTM standards have been established for water activity in cannabis. These include:

1. ASTM D8916-18 – Standard Practice for determining water activity
2. ASTM D8197-18 – Standard for maintaining water activity
3. ASTM Standard in Progress – Stability Testing

WATER ACTIVITY AND MICROBIAL SAFETY

Each microorganism has an ideal internal water activity and their ability to reproduce and grow depends on maintaining that water activity. When a microorganism encounters an environment where the water activity is lower than their internal water activity, they experience osmotic stress and begin to lose water to the environment since water moves from high water activity (energy) to low water activity. This loss of water reduces turgor pressure and retards normal metabolic activity. To continue reproducing, the organism must lower its internal water activity below that of the environment. It tries to achieve this by concentrating solutes internally. The ability to reduce its internal water activity using these strategies is unique to each organism. Consequently, each microorganism has a unique limiting water activity below which they cannot grow [1, 2]. An organism's ability to reproduce and grow does not depend on how much water is in its environment (moisture content), only on the energy of the water (water activity) and whether it can access that water for growth. A list of the water activity lower limits for growth of common spoilage organisms can be found in Table 1. These growth limits indicate that all pathogenic bacteria stop growing at water activities less than 0.87 while the growth of common spoilage yeasts and molds stops at 0.70 aw, which is known as the practical limit. Only xerophilic and osmophilic organisms can grow below 0.70 aw and all microbial growth stops at water activities less than 0.60. In addition, microbial growth rate can be modeled using water activity, along with other growth factors such as temperature and pH. For a cannabis product to be considered shelf stable, its water activity must be less than 0.86 aw to ensure that no pathogenic bacteria will be able to grow on the product as it sits on the shelf. Cannabis products with a water activity higher than 0.70 aw but less than 0.86 aw are considered shelf stable but will still support the growth of mold and yeast. Cannabis products in this range are not considered unsafe because while possibly undesirable to the consumer, molds and yeasts do not cause foodborne illnesses. However, even the growth of non-pathogenic organisms can result in the production of mycotoxins and aflatoxins, which can be harmful if consumed, but especially if inhaled. Consequently, the water activity must be reduced to below 0.70 aw or other interventions such as a preservative system or vacuum packing must be used to prevent mold growth.

Microorganism	aw limit	Microorganism	aw limit
Clostridium botulinum E	0.97	Penicillium expansum	0.83
Pseudomonas fluorescens	0.97	Penicillium islandicum	0.83
Escherichia coli	0.95	Debarymyces hansenii	0.83
Clostridium perfringens	0.95	Aspergillus fumigatus	0.82
Salmonella spp.	0.95	Penicillium cyclopium	0.81
Clostridium botulinum A B	0.94	Saccharomyces bailii	0.8
Vibrio parahaemolyticus	0.94	Penicillium martensii	0.79
Bacillus cereus	0.93	Aspergillus niger	0.77
Rhizopus nigricans	0.93	Aspergillus ochraceus	0.77
Listeria monocytogenes	0.92	Aspergillus restrictus	0.75
Bacillus subtilis	0.91	Aspergillus candidus	0.75
Staphylococcus aureus (anaerobic)	0.9	Eurotium chevalieri	0.71
Saccharomyces cerevisiae	0.9	Eurotium amstelodami	0.7
Candida	0.88	Zygosaccharomyces rouxii	0.62
Staphylococcus aureus (aerobic)	0.86	Monascus bisporus	0.61

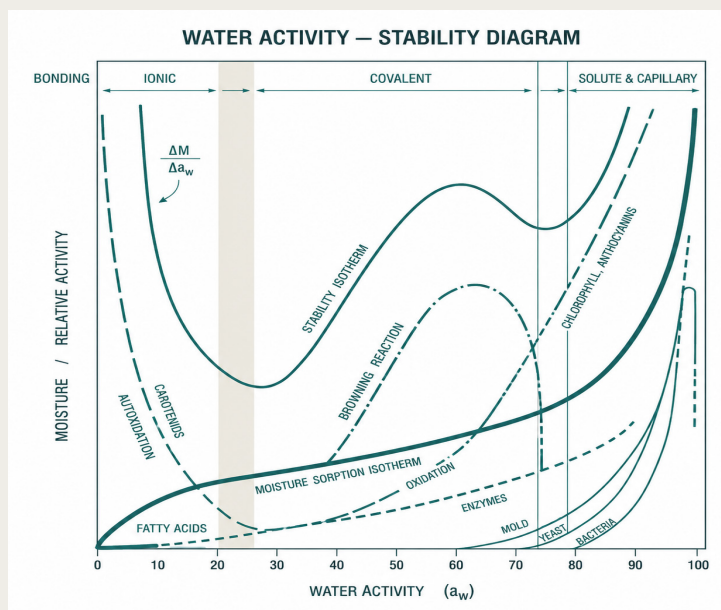
Table 2. Water activity lower limits for growth for common spoilage organisms

WATER ACTIVITY AND CHEMICAL STABILITY

If cannabis biomass and edibles are processed to water activities less than 0.70 aw, microbial spoilage is no longer the most likely mode of failure. However, products in this range do not have unlimited shelf life. So what other modes of failure are likely to occur to end shelf life? For cannabis biomass or edibles in the 0.40-0.70 aw range, chemical degradation is a strong candidate because reaction rates are at a maximum. Chemical reactions such as Maillard browning, lipid oxidation, enzymatic, and others can affect the taste, appearance, and nutritional value of biomass or edibles. Water activity influences reaction rates by reducing activation energy, increasing mobility, and increasing the rate constant. Consequently, reaction rates are better correlated to water activity than moisture content. In general, as water activity increases so do reaction rates, but specific correlations depend on the type of product and the reaction (Figure 1). Most reactions will reach a maximum in the range of 0.70-0.80 aw due to dilution at high water activities, but lipid oxidation is the only reaction that increases at low water activity. For marijuana, the reaction most likely to impact its quality is THCA loss due to decarboxylation, which will reduce its potency. For cannabis edibles, the reaction that is most likely to impact the quality is Maillard browning for products containing protein and reducing sugars, or lipid oxidation (rancidity) for samples containing high levels of fat. These reactions are complex and cause problems through the production of odor and flavor compounds. When the reaction has progressed to produce enough undesirable compounds or loss of THCA, the products will become unacceptable to consumers. The impact of chemical reactions can be minimized by limiting reactants such as reducing sugars for browning or oxygen for rancidity and decarboxylation. Lipid oxidation is unique in that its rate not only increases as water activity increases, but it also increases at low water activity making the general rule that lower water activity is better not true in all cases. To aid in determining the ideal water activity for slowing down chemical degradation, the reaction rate can be predicted using shelf life models. To be effective, these models need to account for the effect of water activity and temperature. The only fundamental shelf life model that includes both water activity and temperature is hygrothermal time [2]. It is derived from a form of the Eyring [3] equation for rate change and Gibbs equation for free energy and is given by

$$r = r_0 \exp \left(B a_w - \frac{E_a}{RT} \right)$$

where T is the temperature (K), R is the gas constant (J mol⁻¹ K⁻¹), Ea is the activation energy (J mol⁻¹), B is the molecular volume ratio, aw is the water activity, and r0 is the rate at the standard state. In practice, the values for B, Ea/R and r0 will be unique to each situation and are derived empirically through least squares iteration. Once the constants are known, any temperature and water activity can be used with the hygrothermal time model to determine rate of change at those conditions and hence the shelf life for a particular product, as it relates to that change. This model can be used to establish the ideal water activity where chemical degradation is at a minimum for cannabis biomass or edibles, thereby maximizing shelf life. The identification of and processing to this ideal water activity that prevents microbial growth while minimizing chemical degradation and texture is the key to maximizing the shelf life of cannabis products.



WATER ACTIVITY AND STORAGE STABILITY

Harvested cannabis (marijuana and hemp) must be sufficiently dry to allow for storage and transport. As explained previously, water activity determines if molds, yeasts, or bacteria will be able to grow on biomass during storage. Dried biomass will typically have a water activity in the 0.60 to 0.70 a_w range. While the growth of pathogenic bacteria would pose the greatest risk at the typical water activity of biomass, the more likely contamination source will be molds. All molds except a few rare xerophilic species stop growing at water activities less than 0.70. While molds themselves are not particularly dangerous if consumed, molds also can produce mycotoxins as part of their metabolism and these can cause severe reactions in some people. In addition, the presence of actively growing mold also means the presence of mold spores. This can be particularly dangerous for a product that is inhaled, resulting in mold spores in the respiratory tract which can lead to asthma symptoms. Consequently, the water activity of any harvested biomass being stored or transported needs to be below 0.70 a_w . This means that water activity testing needs to begin with cultivators and processors. The water activity of edible cannabis products can also change during storage and transport. Baked edibles will have particularly high-water activities and must be tested to make sure they are processed to water activities less than 0.86 a_w to prevent the growth of pathogenic bacteria. Then, their water activity must be maintained in the ideal water activity range during storage to both prevent an increase in water activity to unsafe levels, as well as a reduction in water activity that could lead to undesirable changes in texture. To prevent this, edibles need to be packaged in good moisture barrier packaging. The exact packaging requirements to prevent changes in water activity can be modeled and predicted, but the derivation and use of these models is beyond the scope of this paper.

THE MOST IMPORTANT SPECIFICATION

Water activity plays a key role in ensuring the safety of cannabis products and maximizing shelf life. Water activity may be a new concept to many in the cannabis industry, and those familiar with water activity may only know of its ability to control microbial growth. However, in many cases, microbial spoilage is not the most likely mode of failure for the shelf life of cannabis products. Water activity is related to all common modes of failure and consequently may be the most important test that can be run on everything from harvested biomass to edibles. If you are interested in learning more about how to maximize the effectiveness of your water activity testing, please contact Dr. Brady Carter.

The Author

Dr. Brady Carter is a Senior Research Scientist with Carter Scientific Solutions. He specializes in Water Activity and Moisture Sorption applications. Dr. Carter earned his Ph.D. and M.S Degree in Food Engineering and Crop Science from Washington State University and a B.A. Degree in Botany from Weber State University. He has 20 years of experience in research and development and prior to starting his own company, he held positions at Decagon Devices and Washington State University. Dr. Carter currently provides contract scientific support to Novasina AG and Netuec Group. He has been the instructor for water activity seminars in over 23 different countries and has provided on-site water activity training for companies around the world. He has authored over 20 white papers on water activity, moisture sorption isotherms, and complete moisture analysis. He has participated in hundreds of extension presentations and has given talks at numerous scientific conferences. He developed the shelflife simplified paradigm and hygrothermal time shelf life model.



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