The Creation of an Inexpensive Water Activity Meter and The Effects of Water Activity on Growth of *Rhizopus stolonifer*

Aram Baghdassarian
William A. Shine Great Neck South High School
May 2015

Abstract

Water activity is a measure of the unbound water in a substrate. Microorganisms are able to use this water for growth, meaning water activity can indicate the likelihood of food spoilage. Accurate but expensive ($1000+) water activity meters are typically used by large food production plants. The objective was to build a cost-effective device ($20) to measure water activity and observe its effects on the growth rate of *Rhizopus stolonifer*. The device was built with the Arduino Uno and calibrated with various salt solutions of known water activity. Mold was grown in different water activity levels to see the effects of water activity on mold growth. Water activity had a bell-shaped relationship with mold growth: extreme water activities decreased mold growth. The results can be used to determine proper storage conditions to maximize shelf-life.

Introduction

Household food spoilage is a problem present all throughout the world. According to a USDA-Economic Research Service study, 18.9 billion pounds of fresh fruits and vegetables were lost annually due to spoilage, which was 19.6% of all US losses of edible foods that year (Kantor, L. S., Lipton, K., Manchester, A., & Oliveira, V, 1997).

Another downside of food spoilage is the food borne illnesses it may lead to. It is estimated that just 34 major pathogens cause 9.4 million foodborne illnesses annually in the U.S. (Scallan et al., 2011). In a more specific study carried out by Fields, Zamora, and Bradsher, it was found 116 out of 292 containers of home-canned tomatoes and 41 out of 290 containers of home-canned green beans contained viable organisms and conditions for the growth of

neurotoxin producing *Clostridium botulinum* (Fields, Zamora, & Bradsher, 1977). If homeowners are able to measure the water activity of the environment inside a container holding food, then they will be able to tell if growth of *Clostridium botulinum* is likely and store the containers in cooler temperatures if need be.

Moisture content is a measurement used to determine the shelf-life of foods in some cases, but is less effective in doing so than water activity (Mathlouthi, 2001). One popular method of determining moisture content is by obtaining the mass of the object before and after vacuum drying it (Karel & Fennema, 1975). The issue present with moisture content is that it measures the total amount of water, not just the bound water (Mathlouthi, 2001). Therefore, moisture content is not the ideal measurement to use when one desires to measure the amount of water present to take place in a chemical reaction. Therefore, this also means that moisture content is not useful for finding a value where microbial growth is limited. Instead, water activity is used for this purpose.

Water activity is a measure of the ability of the water inside a food to take part in a chemical reaction (U.S. Food and Drug Administration, 1984, April 16). For example, bread has a high water activity while General Mills Cheerios generally have a low water activity (Mathlouthi, 2001). Therefore, bread is more likely to participate in chemical reactions and spoil faster than Cheerios. Mathematically speaking, water activity is the ratio of the vapor pressure of the water in a substrate to that of pure water at the same temperature (U.S. Food and Drug Administration, 1984, April 16). For example, if the majority of the water is bound to a protein molecule, then the water activity will be reduced since there is less free and unbound water (Mathlouthi, 2001).

Water activity is one of the most common methods of moderating microbial growth (Chirife & Favetto. 1992), and can be useful in preventing food spoilage. Each species of microorganisms has a limiting water activity level below which its growth will be inhibited (Abraham et al., 2004). The FDA includes water activity as a measure of safety by stating that a "potentially hazardous food" does not include a food with a water activity value less than .85 (Abraham et al., 2004). In other words, if a food has a water activity of greater than .85, then it is potentially hazardous.

Many other studies focus on finding the water activity below which a certain organism will not grow. For example, Medveďová et al. researched the effect of altering the water activity on the growth of *Staphylococcus aureus* (Medvedova, Valik, & Studeničová, 2009). They found that this bacteria generally grows best at a water activity of .988 in conditions of 15°C and 18°C. Another study found that the fungus *Penicillium roqueforti*, known for decaying organic matter, can only grow at water activities above .92 (Valık, Baranyi, & Görner, 1999). Bekada et al. studied how water activity, temperature, and pH can limit the growth of the allergenic mold *Mucor racemosus* (Bekada, Benakriche, Hamadi, & Bensoltane, 2008). It was found that growth of the mold was optimal at a pH of 6, water activity of .987, and a temperature of 25°C.

Some researchers have gone so far as to create equations modeling microbial growth that incorporate water activity as one the variables. One such equation models E. coli as a function of temperature, lactic acid concentration, pH, and water activity (Presser, Ross, & Ratkowsky, 1998). Water activity has near equivalent impact in this equation as the other variables (temperature, pH, lactic acid concentration), for the equation is essentially subtracting the minimal water activity, pH... from the actual value. Another equation was developed to model the growth of *Botrytis cinera* as a function of water activity and temperature (Lahlali, R.,

Serrhini, M. N., Friel, & Jijakli, 2007). The impact of temperature alone on fungal growth had an F-value around 9,000 while water activity's impact on fungal growth had an F-value of around 20,000. The study found that at 25°C, growth of B. cinera was greatest at a water activity of .987. Another study related the growth rate of *Aspergillus niger* to water activity and temperature (Parra & Magan 2004). The optimal water activity for growth of *Aspergillus niger* at 25°C was .97, and the minimum water activity necessary for growth at that same temperature was .82.

Rhizopus stolonifer (black bread mold) is one microorganism that can be devastating to post-harvest grains and fruits if not controlled (El Ghaouth, Arul, Asselin, & Benhamou, 1992). *R. stolonifer* is known for decaying harvested or over-ripe stone fruits, along with causing postharvest disease on many fruits and vegetables (Stevens et al., 2004). Stone fruits, *R. stolonifer*'s prominent victims, are fruits with some sort of flesh or pulp enclosing the stone. They are characterized by a soft, watery rot and are fast growing in a wide array of temperatures and relative humidity values (Nishijima, Ebersole, & Fernandez, 1989). Additionally, this fungus is the second largest threat to spoiling postharvest peaches (Ogawa, Lcornard, Manji, Bose, & Moore, 1971). Yet the growth of this mold can be inhibited if put under the right conditions.

A relatively inexpensive device to measure water activity would be beneficial to homeowners and small stores. The devices currently available today to measure water activity cater to the extreme accuracy necessary to manufacturers. The chilled-mirror method utilizes an internal fan, an infrared temperature sensor, an optical sensor, and a mirror. This method works by measuring the dewpoint and surface temperature the instant that condensation begins to form on the mirror (Revercomb et al., 1998). Due to this convoluted method, the cost of water activity meters tend to range from the hundreds to the ten thousands. For example, the Pawkit Water Activity Meter from Aqua Lab costs approximately $2,000 and is the cheapest meter found on

their website, aqualab.com. For household use, a less accurate device would suffice, since there are no strict regulatory standards needed to be met. Once they know the water activity of the food, they can adjust it by changing the temperature or drying the food (Karel & Fennema, 1975) to help prevent foodborne illnesses.

The aim is to develop a relatively inexpensive and sufficiently accurate device to measure water activity in the household, in addition to determining the device's effectiveness by measuring the water activity of the environment and seeing how it affects the growth of *Rhizopus stolonifer*. The device must be sufficiently accurate as to avoid having readings that would indicate an organism cannot grow in an environment when it in fact does have the capacity to do so.

Materials and Methods

Hardware and Software

The materials used to construct the device were purchased primarily from Jameco and Adafruit, with the exception of the Arduino Uno microcontroller from Banggood. Additional materials include the DHT22 temperature and humidity sensor, 10kΩ pull-up resistor, 16x2 LCD display, 50kΩ potentiometer, male pin strip headers, and a strip board. Figure 1 illustrates the appearance of the device. The total cost for the entire device was $19.03

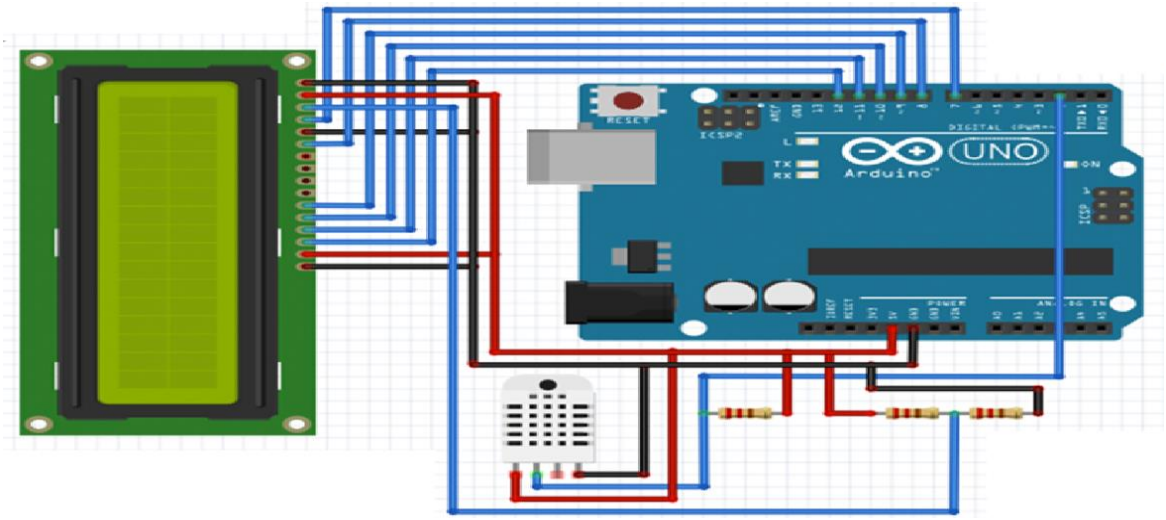

Figure 1: A schematic of the complete device, including the LCD, DHT22 and Arduino Uno.

Additionally, a 9-volt battery could be used to power the device in place of a computer. Using a battery as a power source instead of a computer meant that the data could only be displayed on a LCD with 2 rows of 16 columns (1 column per character) each. However, using a battery is a much more convenient and realistic method of powering the device.

The computer library used to receive and interpret the DHT22 readings was provided by Robert Tillard (2011), and the program was run using the Arduino IDE. Readings were obtained with the Arduino IDE, where the temperature and humidity from the DHT22, time, and calculated water activity were received every two seconds.

Calculation of Water Activity

Two methods of calculating water activity were implemented in this study. The vapor pressure method is calculated by dividing the vapor pressure of water in a material by the vapor pressure of pure water (Murphy & Koop 2005). This could be calculated with solely the relative humidity and temperature but does require an environment at equilibrium.

Equilibrium Relative Humidity (ERH) is found by dividing the relative humidity by 100 when the environment is in equilibrium. ERH is equivalent to water activity.

When obtaining readings, it was found that both methods produced extremely close results, with the only variation appearing in the thousandths place.

Calibration

A 300 ml plastic bottle was used to hold salt solutions. A hole was drilled through the lid of the bottle, and the wires of the DHT were threaded through this hole so that the DHT sensor would be hanging inside and near the top of the bottle while the wires were connected to the Arduino Uno Board outside of the bottle. Parafilm was used to seal the holes in between the threaded wires and lid in order to ensure that the equilibrated atmosphere was maintained.

Composition of Salt Solutions

a_w	Composition
1.00	Distilled water
0.94	1.71 M NaCl
0.90	3.26 M NaCl
0.86	3.76 M NaCl
0.76	6.00 M NaCl
0.59	8.80 M NaBr
0.5	8.57 M LiCl

Table 1: The composition of solutions of differing water activities. 50 ml of each solution was used during calibration

The compositions of salt solutions of specific water activity (Table 1) were retrieved from Barbosa-Canovas et al. 50 ml of each solution were placed into a bottle with a volume of roughly 300 ml (Barbosa-Canovas, Fontana, Schmidt, & Labuza, 2007). The solution was allowed to equilibrate within the bottle for 24 hours, and water activity readings were taken after this time period.

The values received from the device for each water activity were plotted against the actual water activities, and a line of best fit was created. The slope of the line of best fit was taken and used as a calibration equation, where the water activity received is inputted and returns a more accurate calibrated value for water activity.

Mold

R. stolonifer stock culture was obtained on a petri dish from Carolina Biological Supply Company. The mold was incubated at 23°C. All samples of *R. stolonifer* were grown on potato dextrose agar (PDA)

The PDA was created with 250 grams of unpeeled potatoes, 20 grams of agar, 10 grams of anhydrous dextrose, and 1.5 grams of yeast extract. Separate batches of PDA were used for the two trials of the experiment.

Inoculation of new plates involved a solution containing a mixture of fungal spores and sterile water. The spores were suspended in sterile water so that the transmittance was 90% at 600nm. Before inoculating the plates, the mixture was vortexed to ensure that the spores were evenly dispersed. It was found that if the plates were inoculated with only 1 µl of this mixture, then the mold would cover the plate in one or two days. Therefore, three diluted solutions were created: 10x, 100x, and 1,000x dilution. The suspension diluted 100-fold produced mold that took a week to cover the plate, and was used in the experiment. Additionally, it was found that at 18°C, the mold did not grow to fill the plate in just one or two days. Resultantly, the treatment groups were incubated at 18°C. 2µL aliquots were added to the center of each petri dish when inoculating.

In the experimental setup, the sandwich container was placed upside down, and two Petri dishes were taped diagonally to the lid. Two Drosophila vials were cut to a height of 4cm, taped to the lid, and filled with 15mL of the salt solution of the treatment group. Six treatment groups were tested: 0.94, 0.86, 0.76, 0.69, 0.59, and 0.5. These water activities were chosen due to their diversity in the range of values they cover. Furthermore, no solution was needed below .5 due to the fact that it is difficult for microorganisms to grow at lower water activities. In order to measure the growth of the mold accurately without opening the sandwich container and ruining

the equilibrium, paper rulers were created and taped to the transparent container. Photos were taken daily of each container, and Photoshop CS6 was used to overlay the rulers on top of the petri dishes and measure the growth in that way.

Mold growth was checked daily. The sandwich containers were taken out of the incubator and a picture was taken from an aerial view. The orthogonal diameters of each plate were taken and diameters of each box were averaged in order to produce one mean value of growth for each water activity.

Results

Overview

A device was created to measure water activity using a DHT22 relative humidity and temperature sensor. The water activity was obtained by dividing the relative humidity at equilibrium by 100. Saturated salt solutions of known water activities were used to calibrate the device after 50 ml of the solution were left to equilibrate in a 300ml bottle for 24 hours.

The effects of water activity on the growth of *Rhizopus stolonifera* were also measured. Six treatment groups of water activities ranging from .94 to .5 were included.

Calibration

Before calibration, the results strayed a significant amount from the actual (Figure 2). The uncalibrated values appeared to become less accurate as the water activity declined. In addition, the precision of the device was not excellent. For example, in the .90 group in Figure 1, the lowest water activity was .72 and the highest read water activity was .9.

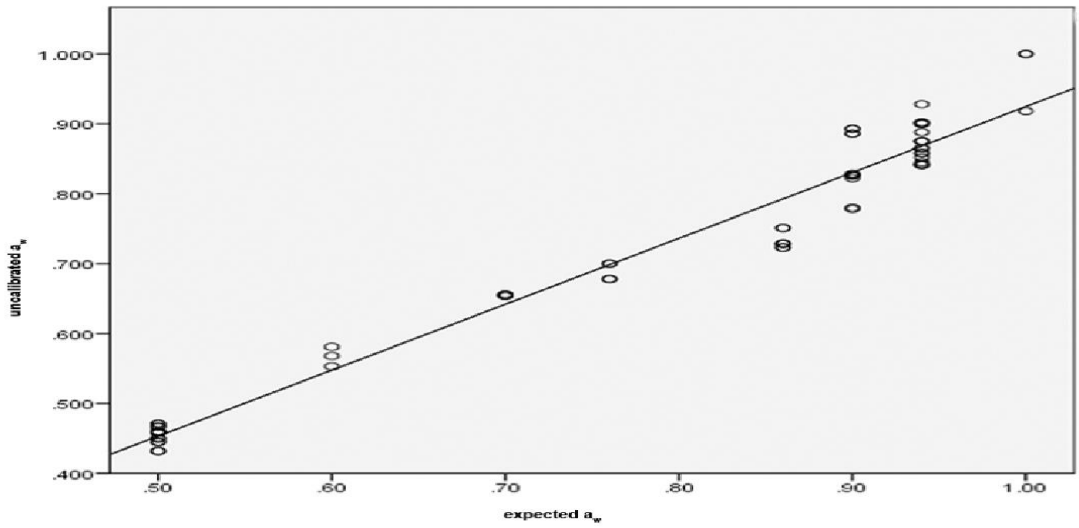

Figure 2: The graph of the expected water activity vs. the uncalibrated value received from the device. A line of best fit runs through the graph.

The equation for the line of best fit in Figure 2 was taken and used to produce values for Figure 3a by entering uncalibrated values into the equation and graphing them against the expected water activity. The equation for the line is y=1.06x+.019, where y is the calibrated water activity and x is the uncalibrated (original) value. The line in Figure 3a is y=x, indicating that any point on the line displays a 1:1 relationship between the expected and calibrated water activities.

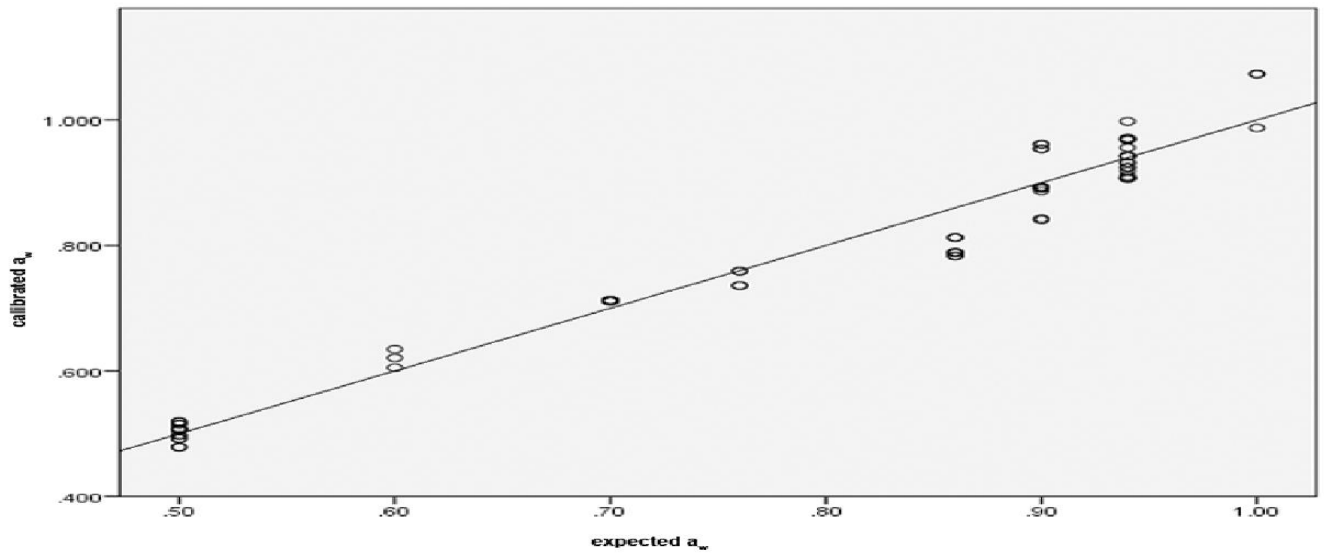

Figure 3a: A graph of the expected water activity vs. the calibrated water activity values. The line in this graph is y=x.

Caused by the formula for the calibration, there is one impossible point on this graph that indicates a water activity above 1.00. This issue of impossible values was fixed by outputting 1.00 if any value was above 1.00 after the calibration.

Figure 3b shows a less cluttered version of Figure 3a by only displaying the mean of all the data points in a group.

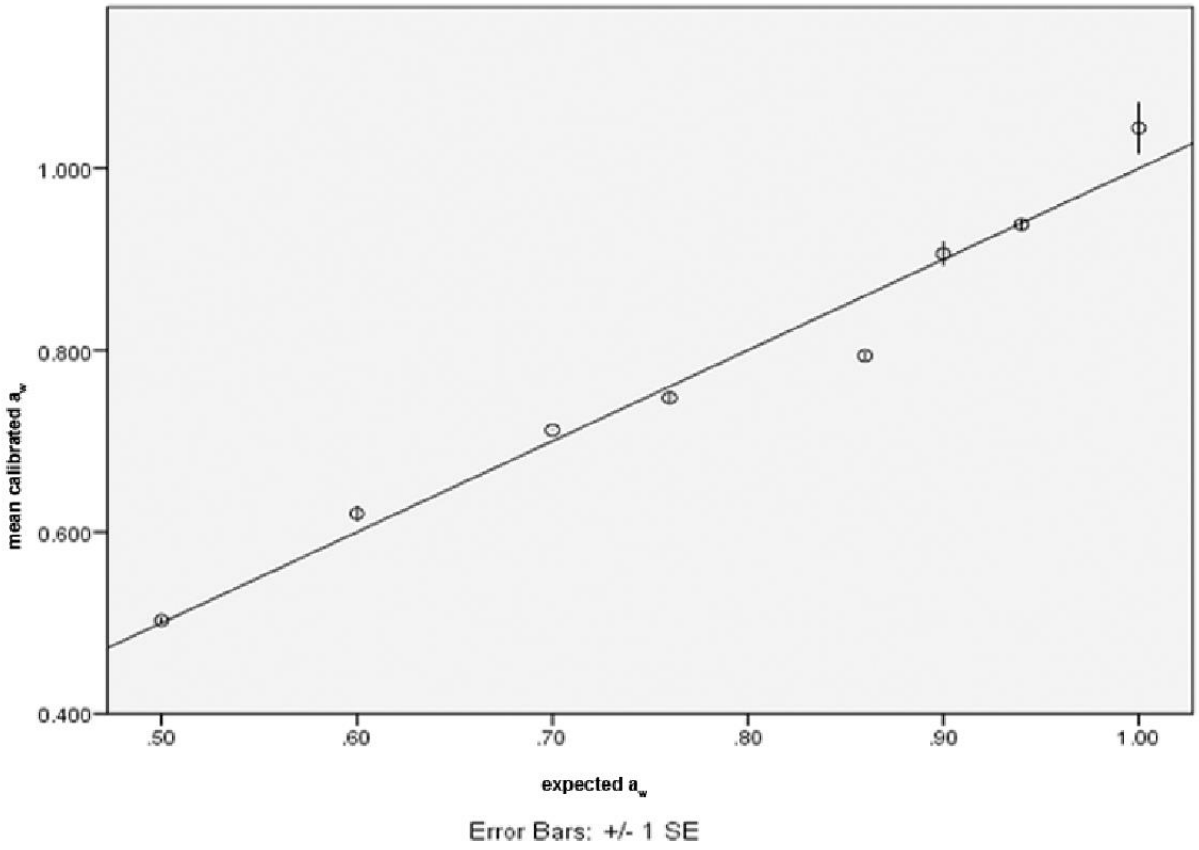

Figure 3b: A graph of expected water activity vs. calibrated water activity, with each point being a mean of all the values in that group

Additionally, it was found that the groups at water activities of 1.00 would equilibrate and output the correct value in a matter of minutes. .94 sometimes experienced this trait too, but no other groups exhibited similar behavior.

Mold

The growth of the mold peaked at .76, and dropped at .69 as seen in Figure 4. If the outlier of .69 is omitted, then the graph is close to having a parabolic form. Only one trial spanning five days for all six groups was conducted.

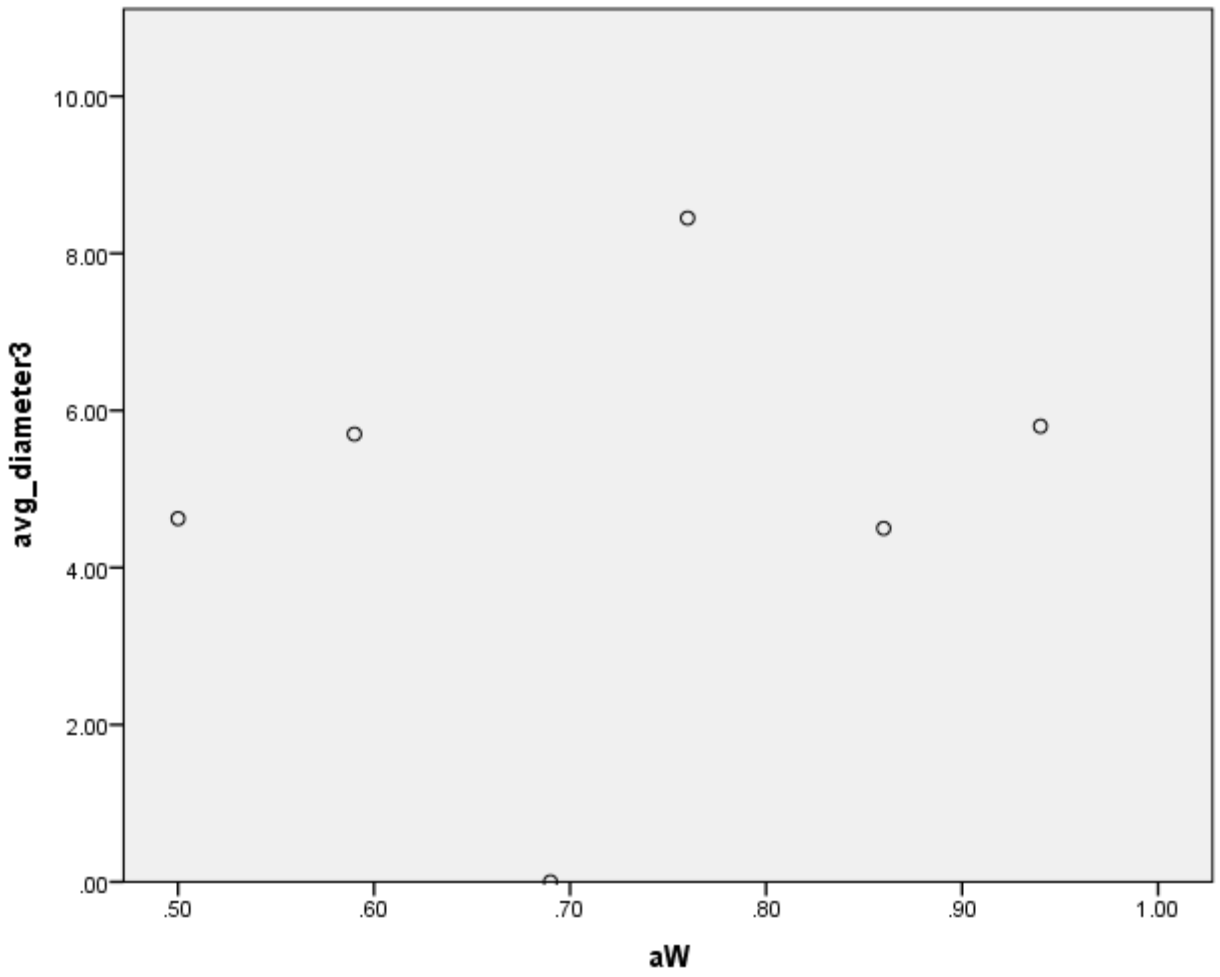

Figure 4: A graph showing water activity vs. the average diameter of the plates of mold on the third out of fifth day of the experiment. P-value <.05 when .69 data point is omitted.

It is important to note that these points are an average of the growth of *R. stolonifer* in two petri dishes per sandwich container. The diameters for each petri dish were not always identical to that of the partner petri dish in the same container and same water activity.

Discussion

In the end, a device was created costing only $20 that can measure the water activity. Due to the relatively low amount of significant figures that this device can achieve when obtaining readings, this is not meant for factory owners who must ensure that their food is of absolute best quality. Instead, this can be of use to home or small store owners who simply would like to know if their food is being stored in a place that is susceptible to spoilage. This means that waste could be reduced through proper storage and maintenance.

The choice of the DHT22 was based off its price and accuracy. The DHT11 was half the price, but would only output integers. The DHT33 and DHT44 were more expensive and did not provide a significant increase in accuracy. The HIH4030 did provide more accurate relative humidity results, but cost 50% more than the DHT22 and did not have the capacity to measure temperature. All the sensors mentioned above can be found on Adafruit.com

The calibration proved to be very successful. Due to the location of the study, it was not possible to do overnight tests to see exactly how long it took for the solution to equilibrate with the environment. The only issue with the device is its precision; however, the only way to resolve this would be to purchase another more expensive device, thus driving up the cost and ruining the purpose of the product.

The mold data is not concurrent with other papers. Other papers previously mentioned show that the mold grew at water activities above .9. A mold growing at a water activity of .76 is rare, and therefore the methods must be examined closely for errors.

During the calibration of the device, two drosophila vials of 30 ml successfully altered the water activity of the environment. It is uncertain as to whether or not the water activity inside the sandwich containers was equivalent to the water activity of the saturated solutions. It was not possible to measure the water activity inside the container without drilling a hole in each

container and having one water activity meter per container. Therefore, it was assumed that the environments would have the right water activity because it worked during the calibration.

However, the most probable cause of the issue with the mold data is the method of changing the water activity of the environment in a way that affects the mold. For example, in Medvedova's study, the water activity was altered by adding a solute to the agar (Medvedova, Valik, & Studeničová, 2009). This would explain why there does not appear to be a definite parabolic pattern for the growth of the mold in this experiment, and why it would peak at such a low water activity opposed to a higher one (it would be random).

The .69 outlier in Figure 4 may be explained by the fact that KI is used as an antifungal (Sterling & Heymann, 2000). Alternatively, the cause may be that the spores in the fungus and sterile water mixture settled while the plates were being inoculated. The solution was not vortexed before each and every individual petri dish, only before the group of 12 petri dishes.

Even with the possible flaw in adjusting the water activity, Figure 4 still exhibited a bell-shaped relationship between growth rate and water activity. Growth rate tended to increase at high water activities but dropped off at higher values. Again, this is not similar to past studies. Another fungus *Botrytis cinera* does not share this relationship (Lahali et al., 2007).

One limitation of the study was the fact that measurement of the mold could only be taken once per day due to classes in school. If readings could be taken multiple times per day, then there would have been a graph with more complete data and more accurate results.

Additionally, due to time constraints, lower water activities such as 0.3 and .4 were unable to be tested in calibration in order to provide a more complete line of best fit.

Future Research

In the future, a more accurate version of the device may be developed. This would be done by repeating the calibration steps mentioned in this paper but rather than using a DHT22 an HIH4030 would be used.

Additionally, the experimental design for the molds would be improved by adding solute directly to the agar instead of just modifying the water activity of the environment. This would likely provide better results for the mold portion of the experiment.

By taking an error and turning it into a discovery, further research could be done on the usefulness of KI as an inhibitor for *Rhizopus stolonifer.*

Lastly, it would be interesting to run the same experiment but with bread instead of Potato Dextrose Agar. The vast majority of studies test the effect of water activity on growth of an organism on agar, rather than on food where the mold is most likely to grow.

Conclusion

For $20, the average household consumer or small store owner is able to have a good idea as to whether their food is going to spoil in the environment it is currently in. The device was calibrated using saturated salt solutions of known water activities. A method and an equation were created from the calibration of the device, making it significantly more accurate. The experiment for the fungus may have been flawed structurally, but a bell-shaped curve relationship was still found between mold growth and water activity. The growth peaked at .76 and declined following that. Another study could be done by adding solute directly to the agar or using foodstuffs in place of agar.

Acknowledgements

I would like to send a large thank you to Dr. Hersh for all the insightful advice she provided about the project. Additionally, none of this would have been possible without the help of Melody Yang and Vivian Lim, my partners in this study.

References

Abraham, A., Al-Khaldi, S., Assimon, S., Beaudry, C., Benner, R., Bennett, R..., & Ziobro, G. (2004). Factors that Affect Microbial Growth in Food. In K. Lampel (Ed.), *The bad bug book: Handbook of Foodborne pathogenic microorganisms and natural toxins handbook*. McLean, Va.: International Medical Pub.

Adafruit Industries, Unique & fun DIY electronics and kits. (n.d.).

Barbosa-Cánovas, G. V., Fontana Jr, A. J., Schmidt, S. J., & Labuza, T. P. (Eds.). (2008). Water activity in foods: fundamentals and applications (Vol. 13). John Wiley & Sons.

Bekada, A. M. A., Benakriche, B., Hamadi, K., & Bensoltane, A. (2008). Modelling of effects of water activity, pH and temperature on the growth rate of Mucor racemosus isolated from soft Camembert cheese. *World J Agricultural Sciences*, *4*(6), 790-4.

Chirife, J., and G. J. Favetto. 1992. Some physico-chemical basis of food preservation by combined methods. Food Res. Int. 25: 389–396.

El Ghaouth, A., Arul, J., Asselin, A., & Benhamou, N. (1992). Antifungal activity of chitosan on post-harvest pathogens: induction of morphological and cytological alterations in Rhizopus stolonifer. Mycological Research, 96(9), 769-779.

Fields, M. L., Zamora, A. F., & Bradsher, M. (1977). Microbiological Analysis of Home- canned Tomatoes and Green Beans. Journal Of Food Science, 42(4), 931-934.

Hernández-Lauzardo, A. N., Bautista-Baños, S., Velázquez-del Valle, M. G., & Trejo-Espino, J. L. (2006). Identification of Rhizopus stolonifer (Ehrenb.: Fr.) Vuill., causal agent of Rhizopus rot disease of fruits and vegetables. *Revista Mexicana de Fitopatología*, *24*(1), 65-69.

Kantor, L. S., Lipton, K., Manchester, A., & Oliveira, V. (1997). Estimating and addressing America's food losses. *Food Review*, *20*(1), 2-12.

Karel, M., & Fennema, O. (1975). Water Activity and Food Preservation. In *Physical principles of food preservation* (2nd ed., pp. 134-5). New York: M. Dekker.

Lahlali, R., Serrhini, M. N., Friel, D., & Jijakli, M. H. (2007). Predictive modelling of temperature and water activity (solutes) on the in vitro radial growth of Botrytis cinerea Pers. *International journal of food microbiology*,*114*(1), 1-9.

Mathlouthi, M. (2001). Water content, water activity, water structure and the stability of foodstuffs. *Food Control,* 409-417.

Medvedova, A., Valik, L., & Studeničová, A. (2009). The effect of temperature and water activity on the growth of Staphylococcus aureus. *Czech Journal of Food Sciences, 27*(Special Iss.(2)).

Murphy, D. M., & Koop, T. (2005). Review of the vapour pressures of ice and supercooled water for atmospheric applications. Quarterly Journal of the Royal Meteorological Society, 131(608), 1539-1565.

Nishijima, W. T., Ebersole, S., & Fernandez, J. A. (1989, June). Factors influencing development of postharvest incidence of Rhizopus soft rot of papaya. In *Symposium on Tropical Fruit in International Trade 269* (pp. 495-502).

Ogawa, J. M., Lcornard, S., Manji, B. T., & Bose, E. Moore. CJ 1971. Monilinia and Rhizopus decay control during controlled ripening of freestone peaches for canning. *J. Food Sci*, *36*, 331-334.

Parra, R., & Magan, N. (2004). Modelling the effect of temperature and water activity on growth of Aspergillus niger strains and applications for food spoilage moulds. *Journal of Applied Microbiology*, *97*(2), 429-438

Presser, K. A., Ross, T., & Ratkowsky, D. A. (1998). Modelling the growth limits (growth/no growth interface) of Escherichia coli as a function of temperature, pH, lactic acid concentration, and water activity. *Applied and Environmental Microbiology*, *64*(5), 1773-1779.

Revercomb, H. E., Feltz, W. F., Knuteson, R. O., Tobin, D. C., van Delst, P. F. W., & Whitney, B. A. (1998, March). Accomplishments of the water vapor IOPs: an overview. In Eighth Atmospheric Radiation Measurement (ARM) Science Team Meeting (pp. 23-27).

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., ... & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*, *17*(7).

Stevens, C., Liu, J., Khan, V. A., Lu, J. Y., Kabwe, M. K., Wilson, C. L., et al. (2004).

The effects of low-dose ultraviolet light-C treatment on polygalacturonase activity, delay ripening and Rhizopus soft rot development of tomatoes. Crop Protection, 23, 551–554.

Sterling, J. B., & Heymann, W. R. (2000). Potassium iodide in dermatology: a 19th century drug for the 21st century—uses, pharmacology, adverse effects, and contraindications. *Journal of the American Academy of Dermatology*, 43(4), 691-697.

U.S. Food and Drug Administration. (1984, April 16). Retrieved from http://www.fda.gov/ICECI/Inspections/InspectionGuides/InspectionTechnicalGuides/ucm072916.htm

Valık, L., Baranyi, J., & Görner, F. (1999). Predicting fungal growth: the effect of water activity on Penicillium roqueforti. *International journal of food microbiology*, *47*(1), 141-146.