Water Activity
An Exploration of Water and Life

Water, water everywhere
Oh how the boards did shrink
Water, water everywhere
Nor any drop to drink

The Rime of the Ancient Mariner – Coleridge

Experiments in Water Activity
Decagon Devices, Inc.

To the teacher:

Each of these lessons is designed to teach valuable concepts based on sound scientific principles.

At the end of these lessons you will find blank lab forms. Students are encouraged to develop their own hypotheses and to actively test them. The lab exercises as included may be used as a supplement to or in lieu of student created labs. The intent was to present a package that could be used 'as is' together with supporting and background materials. These lessons will help your students do science using professional equipment. There is still much science to be done… how many ways will you find to do science with these materials?

You may wish to have your students read Upton Sinclair's The Jungle as a language/literature connection. Food science has developed since the publication of The Jungle. Much of what we know today can be traced back to the need for safer food storage.

Water Quotes

"Water is life's mater and matrix, mother and medium. There is no life without water."
Albert Szent-Gyorgyi, Hungarian biochemist and Nobel Prize Winner for Medicine.

"Water is a very good servant, but it is a cruel master."
C.G.D. Roberts, *Adrift in America*, 1891

"Thousands have lived without love, not one without water."
W.H. Auden

"The noblest of the elements is water"
Pindar, 476 B.C.

"Nothing on earth is so weak and yielding as water, but for breaking down the firm and strong it has no equal."
Lao-Tsze

"If there is magic on this planet, it is contained in water"
Loran Eisley (Anthropologist), *The Immense Journey*, 1957

"When the well is dry, we learn the worth of water."
Benjamin Franklin

"When you drink the water, remember the spring."
Chinese Proverb

"The frog does not drink up the pond in which he lives."
American Indian Saying

"Children of a culture born in a water-rich environment, we have never really learned how important water is to us. We understand it, but we do not respect it."
William Ashworth, *Nor Any Drop to Drink*, 1982

"By means of water, we give life to everything."
Koran, 21:30

Water Activity Quotes

"The single most important property of water in food systems is water activity (a_w) of the food..."
Taoukis P, Breene W, & Labuza TP, *Advanced Cereal Science Technology* 9:91-128 (1988).

"There is now wide agreement that a_w is the most useful expression of the water requirements for microbial growth and enzyme activity. The alternatives of solute concentration and water content have been shown very clearly by Scott (1962) to be inadequate for describing the availability of water for the multiplication of certain bacteria."
Troller JA, & Christian JHB, Water activity - basic concepts. in *Water Activity and Food*, (Academic Press, New York, 1978),Chap. 1, pp. 1-12.

"The practical applications of a_w throughout the food industry have generated creative new food products worth many billions of dollars since the 1960s. Perhaps of even greater potential value is the pool of basic information about a_w and its connection to moisture relations and food qualities that has been generated since the 1960s."
Bone DP, Practical applications of water activity and moisture relations in foods. in *Water Activity: Theory and Applications to Food*, Rockland LB & Beuchat LR, Eds. (Marcel Dekker Inc., New York, 1987), Chap. 15, pp. 369-395.

"It is now generally accepted that a_w is more closely related to the physical, chemical, and biological properties of foods and other natural products than is total moisture content. Specific changes in color, aroma, flavor, texture, stability, and acceptability of raw and processed food products have been associated with relatively narrow a_w ranges."
Rockland LB & Nishi SK, *Food Technology* 34:42-59 (1980).

"There is no single parameter which can be used as a reliable guide to predicting food spoilage or to determining the drying endpoint required for a shelf stable product. The most useful such parameter is water activity (a_w), and much progress has been made in the water relations of foods since water activity research began."
van den Berg C, Water Activity. in *Concentration and drying of foods*, MacCarthy D, Ed. (Elsevier Applied Science Publishers, London, 1986), pp. 11-36.

"The importance of the water activity-moisture content concept of foods cannot be overemphasized. More importantly, the value of water activity has been shown to control the stability of dehydrated and semi-moist foods."
Labuza TP, Sorption phenomena in foods: Theoretical and practical aspects. in *Theory, Determination and Control of Physical Properties of Food Materials*, Rha C, Ed. (D. Reidel Publishing Co., Dordrecht-Holland, 1975), Chap. 10, pp. 197-219.

“Evidence shows conclusively that a_w has a major effect on textural properties of foods.”
Bourne MC, Effects of water activity on textural properties of food. in *Water Activity: Theory and Applications to Food*, Rockland LB & Beuchat LR, Eds. (Marcel Dekker, Inc., New York, 1987), Chap. 4, pp. 75-99.

“Manufacturers should not use the moisture protein ratio (MPR) as a measure of proper drying for shelf-stability or safety. It is product water activity that is best correlated to inhibition of each pathogen’s growth.”
USDA, "Generic HACCP Model for Heat Treated, Shelf Stable Meat and Poultry Products" (2005).

“Water activity plays an important role in the safety, quality, processing, shelf life, texture and sensory properties of foods.”
Fontana AJ & Campbell CS, Water Activity. in *Handbook of Food Analysis, Physical Characterization and Nutrient Analysis*, Nollet LML, Ed. (Marcel Dekker, New York, 2004), Chap. 3, pp. 39-54.

“The water activity principle has been incorporated by various regulatory agencies (FDA CFR 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 AJ, Water activity: why it is important for food safety. in *Proceedings of the First NSF International Conference on Food Safety* 177-185 (1998).

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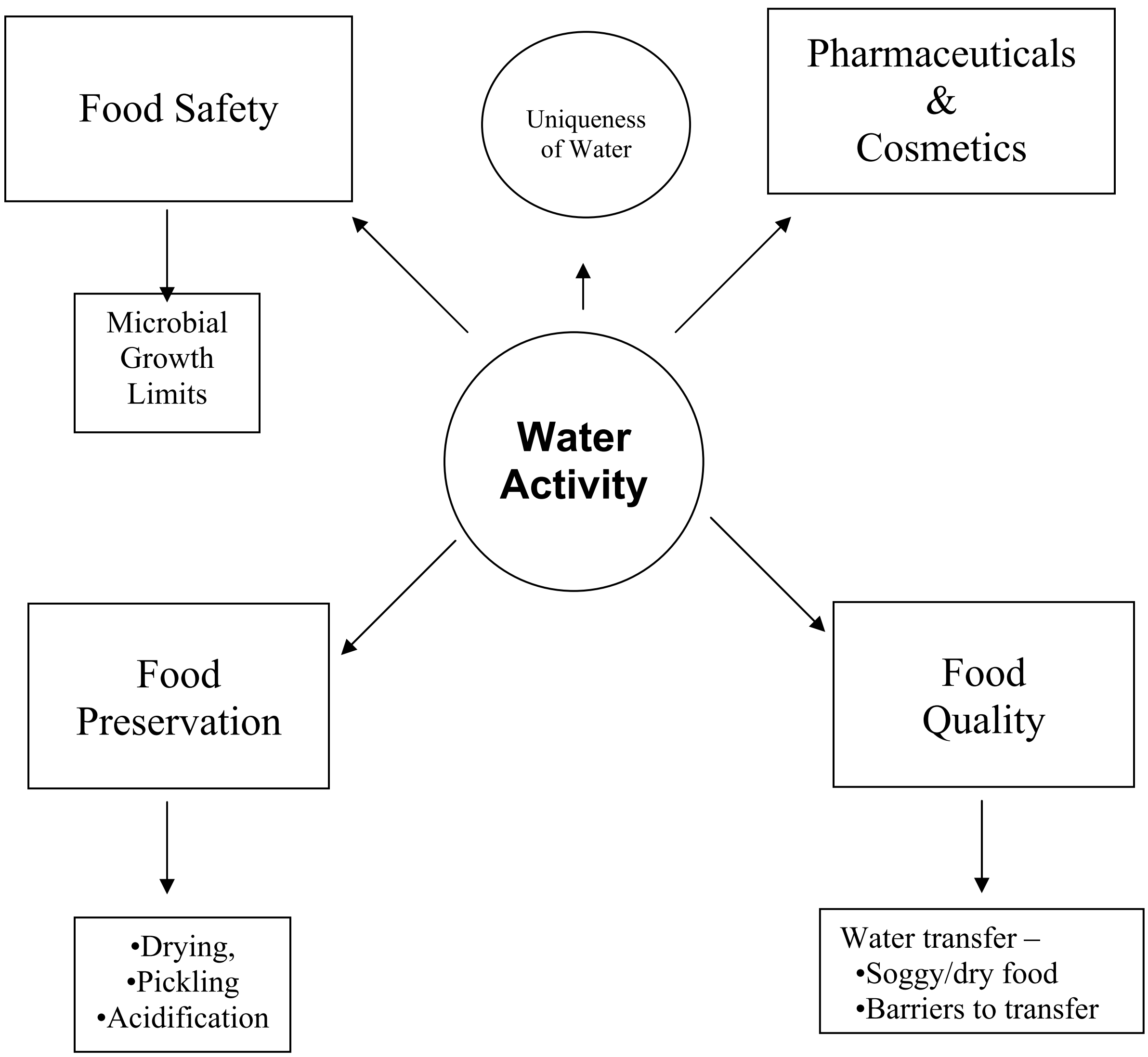
Concept Map


Food Safety
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Water Activity Introduction

Traditionally, discussions about water in products or ingredients focus on moisture or water content, which is a quantitative or volumetric analysis that determines the total amount of water present. Water content of a product is a familiar concept to most people. Moisture content determination is essential in meeting product nutritional labeling regulations, specifying recipes and monitoring processes. However, water content alone is not a reliable predictor of microbial responses and chemical reactions in materials.

The limitations of water content measurement as an indicator of safety and quality are attributed to differences in the intensity with which water associates with other components in the product. The water content of a safe product varies from product to product and from formulation to formulation. One safe, stable product might contain 15% water while another containing just 8% water is susceptible to microbial growth. Even though the wetter product contains proportionally more water, its water is chemically bound by other components making it unavailable to microbes. Using only water content values, it's impossible to know how "available" the water in the product is to support microbial growth or influence product quality.

Water activity is sometimes described in terms of the amounts of "bound" and "free" water in a product. Although these terms are easier to conceptualize, they fail to adequately define all aspects of the concept of water activity. "Free" water is not subjected to any force that reduces its energy therefore all water in food is "bound" water. The issue is not whether or not water is "bound", but how tightly it is "bound". Water activity is a measure of how tightly water is "bound" and related to the work required to remove water from the system. Water that is "bound" should not be thought of as totally immobilized. Microbial and chemical processes are related to this "bound" energy status in a fundamental way.

Water activity describes the energy status or escaping tendency of the water in a sample. It indicates how tightly water is "bound", structurally or chemically, in products. Both the water content and the water activity of a sample must be specified to fully describe its water status. However water activity is the property most relevant for quality and safety issues. Water activity is closely related to the partial specific Gibbs free energy of the system. Thus, water activity is a thermodynamic concept and as such has requirements for measurements. These requirements are that the system be in equilibrium, the temperature defined, and a standard state specified. Pure water is taken as the reference or standard state from which the energy status of water in food systems is measured. The Gibbs free energy of free water is zero and thus the water activity is 1.0.

Water activity is defined as the ratio of the vapor pressure of water in a material (p) to the vapor pressure of pure water (p_o) at the same temperature. Relative humidity of air is defined as the ratio of the vapor pressure of air to its saturation vapor pressure. When vapor and temperature equilibrium are obtained, the water activity of the sample is equal to the relative humidity of air surrounding the sample in a sealed measurement chamber. Multiplication of water activity by 100 gives the equilibrium relative humidity (ERH) in percent.

$$a_w = p/p_o = \text{ERH (\%)} / 100$$

As described by the above equation, water activity is a ratio of vapor pressures and thus has no units. It ranges from 0.0 a_w (bone dry) to 1.0 a_w (pure water).

There are several factors (osmotic, matrix, and capillary) that control water activity in a system. It is a combination of these factors in a product that reduces the energy of the water and thus reduces the vapor pressure above the sample as compared to pure water. Due to varying degrees of osmotic and matrix interactions, water activity describes the continuum of energy states of the water in a system rather than a static "boundness". "Bound" or "free" are not a very useful description since it is an attempt to classify a continuum in terms of discrete states.

There is no device that can be put into a product that directly measures the water activity. Rather, a_w is measured with an indirect method. 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 relative humidity of the headspace. Methods for water activity determinations are detailed in the Official Methods of Analysis of AOAC International (1995). The Pawkit water activity uses the approved capacitance method to determine the water activity of a sample.

Water activity's usefulness as a quality and safety measurement was suggested when it became evident moisture content could not adequately account for microbial growth fluctuations. Water activity is a measure of the energy status of the water in a system. The water activity (a_w) concept has served the microbiologist and food technologist for decades and is the most commonly used criterion for safety and quality. Its usefulness cannot be denied.

Microorganisms have a limiting water activity level below which they will not grow. Water activity, not moisture content, determines the lower limit of "available" water for microbial growth. Since bacteria, yeast, and molds require a certain amount of "available" water to support growth, designing a product below a critical a_w level provides an effective means to control growth. Water may be present, even at high content levels in a product, but if its energy level is sufficiently low the microorganisms cannot remove the water to support their growth. This 'desert-like' condition creates an osmotic imbalance between the microorganisms and the local environment. Consequently, the microbes cannot grow and its numbers will decline until it eventually dies.

While temperature, pH, and several other factors can influence whether an organism will grow in a product and the rate at which it will grow, water activity is often the most important factor. The water activity level that limits the growth of the vast majority of pathogenic bacteria is 0.90a_w, 0.70a_w for spoilage molds, and the lower limit for all microorganisms is 0.60a_w.

Water activity influences not only microbial spoilage but also chemical and enzymatic reactivity. Typically, as the water activity level is lowered the rate of chemical degradative reactions decreases.

In addition to prediction of the rates of various chemical and enzymatic reactions, water activity affects the textural properties of foods. Foods with high a_w have a texture that is described as moist, juicy, tender, and chewy. When the water activity of these products is lowered, undesirable textural attributes, such as hardness, dryness, staleness, and toughness, are observed. Low a_w products normally have texture attributes described as crisp and crunchy, while these products at higher a_w levels change to soggy texture. Critical water activities determine where products become unacceptable from a sensory standpoint.

Because water activity is a measure of the energy status of the water, differences in water activity between components is the driving force for moisture migration as the system comes to equilibrium. Thus, water activity is an important parameter in controlling water migration of multicomponent products. Some foods contain components at different water activity levels, such as filled snacks or cereals with dried fruits. By definition, water activity dictates that moisture will migrate from a region of high a_w to a region of lower a_w, but the rate of migration depends on many factors. Undesirable textural changes can result from moisture migration in multicomponent foods. For example, moisture migrating from the higher a_w dried fruit into the lower a_w cereal causes the fruit to become hard and dry while the cereal becomes soggy

Differences in water activity levels between components or a component and environmental humidity is a driving force for moisture migration. Knowledge of whether water will absorb or desorb from a particular component is essential to prevent degradation, especially if the substance is moisture sensitive. For example, if equal amounts of component 1 at 2% and component 2 at 10% moisture content are to be blended together, will there be moisture exchange between the components? The final moisture content of the blended material would be 6%, but did any moisture exchange between component 1 and 2? The answer depends on the water activities of the two components. If the water activities of the two components are the same, then no moisture will exchange between the two components. Likewise, two ingredients at the same moisture content may not be compatible when mixed together. If two materials of differing water activities but the same water content are mixed together, the water will adjust between the materials until an equilibrium water activity is obtained.

Lesson 1 Is it Safe to Eat?

Hold up a Twinkie® or other food product reputed to have an extremely long shelf life.

<u>Ask students:</u>

Question: If one of these were buried thousands of years ago in a pyramid would it still be fresh today?

Question: What about other foods? Suppose you found a tuna sandwich that had been sitting in the hot sun for several hours—would you consider it safe to eat? Other examples may include undercooked ground beef or foods that contain raw eggs.

Question: Have you ever seen mold on bread? What evidence do you have when food is unsafe?

Answer: Allow time for discussion. As answers are shared help the students generalize that most evidence of food spoilage is sight or smell based.

Question: What about food that looks and smells okay?
Answer: Some bacterial agents can render food harmful without visible or scent evidence. Consider the case of the tuna sandwich left in the hot sun for several hours– it could be quite dangerous and still not 'show' or 'smell' of spoilage.

Question: Since we cannot always tell when a food is unsafe to eat - what is done to ensure the food we eat is safe?
Answer: Allow time for discussion. Good answers may include, but are not limited to:

- Government (food) regulation and inspection
- Refrigeration and/or freezing
- Drying food
- Pickling food
- Canning food
- Cooking food completely
- 'Pull dates' on food products
- Pasteurization
- Preservatives
- Packaging (vacuum packaging or modified atmosphere)

Many of the methods mentioned might be modern but have their origins in traditional methods.

Question: What methods have been used traditionally to preserve food?
Answer: Allow time for discussion. Good answers may include, but are not limited to:

- Salting food[1]
- Sun drying food
- Natural ice caves (when available)
- Sugaring or syruping

Research Project: Research the methods indigenous peoples in your area used to preserve food. Prepare and be ready to present if asked a one to two page summary of the methods and foods used to preserve food for times of scarcity.

Lesson 1 Student Experiment: Preserving Bread

Objective:
To test the effectiveness of one method of preserving bread – drying.
This experiment will take longer to complete than it will to set up so please be patient.

Supplies:

- 2 pieces of bread or one piece divided in half
- 2 sandwich bags (must be able to make airtight, eg ZipLock®)
- Pawkit® Water Activity Meter
- Sample cups (accompanies Pawkit®)

Procedure:
Place one piece of bread in an oven heated to 120°F (or 50° C). You will leave the bread in the oven for about twenty minutes during which time it will become somewhat dry. If you are in a hurry you may use a microwave oven. Wrap the bread in a paper towel and heat it in the microwave for about thirty seconds. Be careful the bread will be hot. Let it cool for a minute or two before touching it. Also, don't overcook the bread; we will do that purposely in a later experiment but not at this time.

Measure the water activity of the dried bread using the Pawkit water activity meter. Place a small piece of the dried bread into a sample cup (be sure to cover most of the bottom of the sample cup). Place the cup into the Pawkit water activity meter. Press button 1 once to turn the Pawkit on and a second time to start the water activity measurement. After five minutes the Pawkit will beep 4 times and the final water activity of the dried bread will be displayed. Next, measure the water activity of the control (un-dried) bread in the same manner.

[1] In some parts of the United States you will find places with the word 'lick' in their names e.g., Boone's Lick or Deer Lick. In these places there was once (and may still be) a 'lick' or natural salt outcropping. These were desirable both as sources for salt (that could be used to preserve food) and for the game that was attracted to the salt.

Place the control (un-dried) bread into a sandwich bag and seal it airtight. This will ensure that moisture will not enter or escape from the bag. Place the dried bread in the other bag and seal it also. Place both bags where they will be warm and where you can inspect them daily.

Inspect each sample for signs of mold at the same time each day. Record the condition of each sample in the table below

	Day 1	Day 2	Day 3	Day 4	Day 5
Dried					
Control					

	Day 6	Day 7	Day 8	Day 9	Day 10
Dried					
Control					

To the Teacher:

If you do not have access to an oven or dehydrator you may substitute plain croutons for the dry bread.

You may wish to have the student perform a slight variation by having them use a third bag with both dry and un-dried pieces of bread.

The timing of mold appearing depends heavily on the age of the bread. Do not be surprised if it takes as little as two or three days or as long as a week to see mold.

Another, possibly interesting, experiment would be to dry the moldy bread thoroughly and see if that stops the mold or, if once started, it continues to grow.

Expected Results and Discussion:

The control (un-dried) bread will show mold growth while the dried bread will not if its water activity has been dried below $0.70a_w$.

Ask the students:

Question: Why did the control bread mold and the dried bread not?

Answer: Most students will say because there is less water in the dried bread than in the control. They are correct, but it is not the amount of water that is controlling mold growth – it is the water activity. By removing water through drying there is not enough 'available' water in the dried bread sample to allow the mold spores to germinate and grow.

Question: Are there mold spores on the dried bread?

Answer: Yes, but because there is not enough 'available' water the mold cannot grow.

Question: Would you see mold growth on the dried bread if it was stored in a humid environment?

Answer: Yes, as soon as the bread absorbed enough water so that there was enough 'available' water the mold spores would germinate and start to grow.

Lesson 1 Student Experiment – Non-molding Bread

Background:

Historical Note:

Hardtack was long use as rations aboard sailing vessels (you can still buy Pilot Bread) and as rations for soldiers in both the Revolutionary and Civil wars. Traditional hardtack was about ½ inch thick and, when several months old, quite hard on the teeth. Union soldiers called hardtack 'sheet iron crackers', 'tooth dullers' or, because they were sometimes infested with weevils, 'worm castles'. In 1861 the 1st Iowa Regiment adapted Stephen Foster's then familiar song "Hard Times Come Again No More" to "Hard Crackers Come Again No More". Here are the lyrics.

Hard Crackers Come Again No More

Let us close our game of poker, take our tin cups in hand,
While we gather round the cook's tent door
Where dried mummies of hard crackers are given to each man;
Oh, hard crackers come again no more!"

Chorus
'Tis the song and the sigh of the hungry,
Hard crackers, hard crackers, come again no more!
Many days have you lingered upon our stomachs sore,
Oh, hard crackers come again no more."

"There's a hungry, thirsty soldier who wears his life away,
With torn clothes, whose better days are o'er;
He is sighing now for whiskey, and with throat as dry as hay,
Sings, "Hard crackers come again no more."
(Chorus)

'Tis the song that is uttered in camp by night and day,
'Tis the wail that is mingled with each snore;
'Tis the sighing of the soul for spring chickens far away,
Oh, hard crackers come again no more."
(Chorus)

But to all these cries and murmurs there comes a sudden hush,
As frail forms are fainting by the door,
For they feed us now on horse feed that the cooks call "mush,"
Oh, hard crackers come again once more."

Final Chorus
It's the dying wail of the starving,
Hard crackers, hard crackers, come again once more.
You are old and very wormy, but we pass you failings o'er,
Oh, hard crackers come again once more."

Traditional bakers had no way of being certain when the bread was 'dry enough' and so they often baked it twice on separate days (Biscotti means 'baked twice'). Today we have the Pawkit Water Activity Meter that can indicate whether the bread is 'dry enough". Simply place a small piece of the bread (at room temperature) in a sample cup and test it with the Pawkit. If the a_w reading is less than 0.6 the bread will not mold, if it is less than 0.4 the bread is 'done'.

While waiting for the bread to mold (we know by experience it will) let's make some old-fashioned 'bread' that will not mold (when properly baked and stored). The 'bread' is called Hardtack (regular bread was referred to as 'Soft Tack')

Hardtack Recipe

<u>Supplies:</u>

- 2 cups of flour (You may wish to use part whole wheat flour)
- ½ cup of water (adjust as needed)
- ½ tablespoon salt (optional)
- ½ teaspoon baking powder (optional -- omitted from authentic hard tack)

<u>Directions:</u>

- Combine all ingredients. Mix until you have very stiff, but not sticky, dough.
- Roll out the dough on a clean surface. When it is about 1/4 to 3/8 inch thick cut into 4" square pieces.
- Using a fork or toothpick make 12-16 holes into each square.
- Place the 'biscuits' onto a cookie sheet and bake in a 350-degree oven for about 1/2 hour (or until it begins to brown).
- Remove from oven and allow to cool
- Serve cold

<u>Objective:</u>

To test the effectiveness of proper storage of Hardtack.

<u>Supplies:</u>

- 2 pieces of Hardtack or one piece divided in half
- 2 sandwich bags (must be able to make airtight, eg ZipLock®)
- Pawkit® Water Activity Meter
- Sample cups (accompanies Pawkit®)
- Moistened paper towel

Measure the water activity of the Hardtack bread using the Pawkit water activity meter. Place a small piece of the Hardtack into a sample cup (be sure to cover most of the bottom of the sample cup). Place the cup into the Pawkit water activity meter. Press button 1 once to turn the Pawkit on and a second time to start the water activity measurement. After five minutes the Pawkit will beep 4 times and the final water activity of the dried bread will be displayed.

Place one piece of Hardtack bread into a sandwich bag and seal it airtight. Place a second piece of Hardtack and a folded paper towel moistened with water into a sandwich bag and seal it airtight. Note: the paper towel should not touch the Hardtack – you are creating a moist (high humidity) environment for the Hardtack.
This will ensure that moisture will not enter or escape from the bag. Place both bags where they will be warm and where you can inspect them daily.

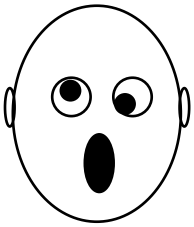
Inspect each sample for signs of mold at the same time each day. Record the condition of each sample in the table below

	Day 1	Day 2	Day 3	Day 4	Day 5
Dried					
Control					

	Day 6	Day 7	Day 8	Day 9	Day 10
Dried					
Control					

Note: You may also have the students test the texture change in the Hardtack during storage by gently pressing on the top of each piece of bread.

Lesson 2 Water Activity and Microbes



"There are 'germs' in my food," says one. "Look at the jar of honey. It clearly states that honey should not be fed to infants under the age of one year. What can I eat?"

"Wait a minute--" comes the reply "let me see that. The label reads: 'Honey should not be fed to infants less than one year of age. Honey is a safe and wholesome food for older children and adults.' That just means don't give honey to small children, perhaps their immune system is still developing. Honey *is* safe for you to eat you're forty years old"

Question: Is there bacteria and fungi in and on the food we eat?
Answer: Before we answer that, remember that food in America is among the safest, if not *the* safest, in the world. When it is properly cared for, cooked and served there is no reason to expect anything else. The object in food safety is not to eliminate all bacteria (some are beneficial), but to limit populations of bad bacteria well below the levels of safety.

Question: Where are bacteria and fungi beneficial?
Answer: Some examples of beneficial agents include:

- Bacteria in the intestine - both the large and small intestines are home to beneficial bacteria. Several brands of probiotics are advertised to help maintain healthy intestinal flora
- Yogurt is the result of bacterial action
- Wine and beer require yeast to ferment
- Naturally occurring bacteria in some foods compete with pathogenic forms (such as *Staphylococcus aureus*), limiting their populations
- Bread
- Cheese manufacturing

Not all microbes are beneficial. The table below lists some agents and the products they may affect.

Microbial Group	Example	a_w	Products Affected
Bacteria (pathogens)	*Salmonella, E.coli, Listeria, Clostridium botulinum*	0.91	Fresh meat, milk
Normal yeast	*Saccharomyces cerevisiae, Candida*	0.88	Fruit juice concentrate
Normal molds	*Aspergillus spp. Penicillium spp.*	0.80	Jams, jellies
Halophilic bacteria	*Halobacterium spp.*	0.75	Honey
Xerophilic molds	*Wallemia spp.*	0.65	Flour
Osmophilic yeast	*Saccharomyces rouxii*	0.60	Dried fruits

Lesson 2 Demonstration: Exponential Growth

Supplies:
You will need a large bag of M&M® or Skittles® candies (or something similar[2]) for this demonstration. The candies must have a distinctive side, such as the 'm' or 's' printed on one side of the candies.

Procedure:

1. Empty the bag of candy into a bowl. This is the reservoir.
2. Make three piles of two candies each:
   Pile 1 – represents a totally hostile environment with not enough 'available' water. The population (2) is static. Perhaps the population exists as spores or organisms that reproduce at zero population growth.
   Pile 2 - represents a less hostile environment with below optimum 'available' water. The growth of these organisms is less than optimal, as sometimes it may double but other times there may be no increase in the number of organisms.
   Pile 3 - represents an ideal environment.
3. Exponential Growth:
   Pile 1 - add 0 candies.
   Pile 2 - shake and drop the candies onto the table surface. For every candy with the letter 'm' or 's' upward add a new candy from the reservoir to Pile 2. For example, if one candy has the letter up and one candy has its letter down – add one candy from the reservoir to this pile.
   Pile 3 – add a number of candies equal to the number already in the pile. For this cycle it would be two candies, for the next it would be four, then eight, etc..
4. Repeat the exponential growth additions until the candy reservoir is depleted

Question: How many tosses will be required before the reservoir is depleted?
(Hint a pound bag of M&M® candy contains approximately 530 candies)

Question: How equal will be the distribution? Which pile will be larger? By how much?

Proceed to demonstrate. It should not take long before the reservoir is totally depleted. Ask the students to comment on the distribution.

Question: What if the reservoir was much larger? Bacterial populations can exceed 100s of millions. How many doublings would be required to achieve 100 million if you started with only 1000 bacteria?

Note: an increase from 1000 to 100,000,000 is a factor of 100,000. Population increases 2^n times where 'n' is the number of doublings. If you solve for $2^n \geq 100,000$ you can determine the number of doublings.

[2] One non-edible option is to use pennies. If you have a large supply of pennies in a jar they are excellent substitutes for candy.

Answer: $2^n \geq 100{,}000$ can be rewritten as log 100,000/log 2 = n   Solving for n yields 16.61, therefore $2^{16.61} = 100{,}000$ or 16.61 doublings are required to increase a population from 1,000 to 100,000,000.

To determine the actual time one must know the doubling time. Under optimum conditions some yeasts and bacteria can double in less than 20 minutes. In non-optimum conditions that time may be extended to several hours. In hostile conditions the organism may not be able to reproduce at all. Maintaining optimum storage conditions including temperature[3], pH and water activity is essential to keeping food safe. Additionally, consumers must use reasonable care in preparing their foods.

Consider the following from the USDA publication *Cooking and Cooling of Meat and Poultry Products* (copies available from the USDA or on the internet)

> **7.1 HEAT STABLE TOXINS**
> Although cooking destroys most vegetative cells of pathogens as well as their toxins, not all toxins are easily destroyed by heat. *Staphylococcus aureus* produces a toxin that results in one of the more economically important diseases in the US. *S. aureus* is very salt tolerant while other foodborne pathogens are not. Because of this tolerance, *S.aureus* can survive well on salted meat products, such as hams and sausages where other organisms cannot compete. It has been found in open sores on the hands and arms of food service employees. The infective dose of *S.aureus* is less than 1.0 micrograms of its toxin, and this level is reached when *S. aureus* population reaches 100,000 organisms per gram in the food. It can produce its toxin at a water activity as low as 0.86. Also, the toxin produced by *S. aureus* is extremely heat-stable, and can survive even boiling or retorting temperatures. Therefore, cooking is not a sufficient barrier to eliminate pre-formed toxin in food. In fact, cooking destroys microbes, which would normally compete with *S. aureus*. The best way to prevent *S. aureus* is through eliminating hand contact with ready-to-eat foods, restricting employees who have infected sores on their hands and arms, and maintaining proper temperatures.

One final word – many common household spices contain innocuous quantities of mycotoxin producing fungi. When added to food before cooking or sprinkled on food shortly before eating these are quite safe but, from what we have seen about fungal growth, it is unwise to add these ingredients, post cooking, to foods that will then be stored for a long time. To discover the greatest potential offenders in your home you will need a growth medium such as pumpkin pie and a sealable plastic bag. Select one slice and sprinkle several types of spices in

[3] Highly publicized past incidences of E. coli being found in non-pasteurized apple juice is one example where low pH alone was not enough to protect the consumer. Modern science has determined the application of several obstacles, or hurdles, including proper water activity can better ensure food safety.

different places. Store the pie at room temperature in a sealed plastic bag. Observe daily for several days. Eventually you will see a strange 'garden' of fungi. Do not open the bag—dispose of bag and contents promptly and properly.

Lesson 2 Student Investigation/ Experiment 1

Testable Question: Can the rate of growth of an introduced microbe be controlled by the type of food in which it grows?

Hypothesis: Yeast requires sugar to grow but not all sugars are the same. Yeast will grow faster in some kinds of sugar than others.

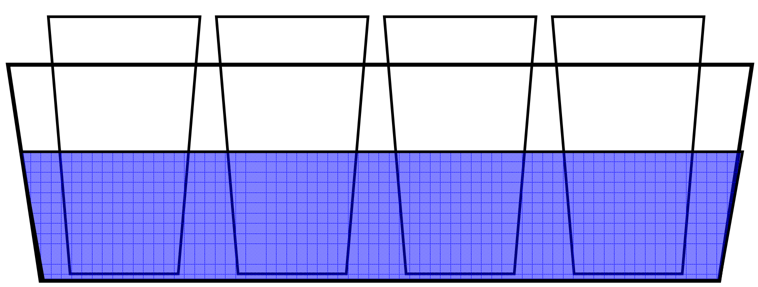
Materials Required:
- Glass jars or cups
- 1 package of active dry Yeast
- Warm water
- Thermometer
- Food for the yeast--
    - Table sugar
    - Corn syrup
    - Molasses
    - Corn starch or wheat flour – a starch that can be converted to sugar

Procedure:

1. Add 100 ml of 45ºC water to each of four glasses. (If you prefer non-metric units use ½ cup water at 110ºF).

2. Open one packet of yeast and add equal amount to each of the four glasses

3. Place the water/yeast containing glasses into a thermal bath— a large water filled container to act as a reservoir of heat to keep the yeast/water mixture at a nearly constant temperature. See the diagram below.



4. To each glass add 1 tbsp of one of the following: sugar, corn syrup, molasses, or starch/flour. Stir gently cleaning the stirrer between each liquid.

Observations:

Which 'food' caused the earliest growth/fermentation?

Which 'food' has the steadiest rate of growth/fermentation?

Can you smell any products of growth/fermentation?

What other evidences can you detect of growth/fermentation?

For Further Investigation: Yeast is a living organism; can the rate of growth be controlled (or growth inhibited) by altering the water activity?
(See next experiment)

Note to the Teacher: Yeast will readily assimilate glucose and fructose. Sucrose, a disaccharide, must first be converted into glucose and fructose before assimilation. Maltose can be formed from starch by amylase but must then be converted first to glucose then to glucose and fructose before assimilation. If some materials, such as corn syrup initially ferment more rapidly than others it is due to the readily available simple sugars in the substance.

Human saliva contains the amylase *Ptyalin* that breaks starch into maltose for further digestion. If you hold a cracker in your mouth long enough you will begin to detect the formation of sugar.

Amylase is synthesized in grain during germination. When cereal grains, especially barley, are sprouted and then quickly dried the maltose remains in the seed and can be harvested as 'malt'.

Lesson 2 Student Investigation/ Experiment 2

Testable Question: Is the growth of an introduced microbe limited by altering the water activity of the food?

Hypothesis: Lowering the water activity by the addition of salt can slow or even eliminate the growth of an introduced microbe (yeast)

Materials Required:

- Pawkit water activity meter with sample cups
- Glass jars or cups
- 1 package of active dry yeast
- Salt
- Warm water
- Thermometer
- Food for the yeast—select one food that worked well in the last investigation
    - Table sugar
    - Corn syrup
    - Molasses
    - Corn starch or wheat flour – a starch that can be converted to sugar

Procedure:

1. Add 100 ml of 45ºC water to each of four glasses. (If you prefer non-metric units use ½ cup water at 110ºF)

2. To each glass add 1 tbsp of the 'food' (sugar, corn syrup, molasses, or starch/flour) that you selected. Stir well.

3. Measure and record the water activity of one 'food' sample.

4. To the next sample add a small amount of salt. Measure and record the water activity of this sample.

5. Repeat for the remaining two samples. Try to get different water activities for each sample with one sample below 0.88. You may have to add additional salt to a glass and then re-test the water activity.

6. Place the water containing glasses into a thermal bath as before. If you have forgotten see the diagram above.

7. When temperature of the water in the glass is stable add equal amount of Yeast to each of the four glasses. Stir gently, cleaning the stirrer between each liquid.

8. Observe the yeast growth in each glass.

Observations:

1. How did the water activity relate to observed growth/fermentation?

2. Was the incidence growth/fermentation different with different water activities?

3. Was growth/fermentation inhibited in any sample?

Questions:

1. There are many ways to prevent the growth of microorganisms one is limiting a_w. In addition to lowering a_w food producers can use other ways to prevent the growth of microorganisms and ensure food safety. Why might a food producer choose to ensure food safety by multiple methods?

For Further Investigation: When bread is being mixed, salt is added. Why? Is it to slow the action of the yeast or is it just for taste? How would bread turn out if salt was omitted? Since bread machines are the most consistent way to make bread – what would happen if salt was omitted from the recipe? How would the no-salt bread compare to the standard recipe. What would be the results?

Lesson 2 Optional Experiment – Making Your Own Malted Barley

- Wash the raw barley to remove chaff (it will float while barley corns will not). Weigh the clean barley (hereafter all references are normalized to 100 grams).
- Drain the barley and place in a covered container with enough water to cover the barley to a depth of 5cm (2 inches)
- Let the raw barley soak for eight hours, drain and let stand for eight hours, then soak for another eight hours in clean water. At this point you should begin to see the *acrospires* (tips) emerging.
- Place the grain on paper towels and place flat inside a large plastic bag. Seal the bag to keep moisture in and dirt out.
- In four to six days the *acrospire* will be approximately 75 to 100% of the length of the grain. The seed is now ready to be placed in a kiln (oven) and should weigh approximately 50% more than when you started. (100 grams of dry barley should now weigh 150 g.)
- Bake the 'green malt' for 24 hours at about 45ºC. The seed should now weigh between 110 and 115 grams.
- Finish baking the grain at 55-60ºC until the weight is about 100 grams. Test the finished grain for water activity to see if it is safe for storage.

- As an alternative try this method for drying the 'green malt'. Spread the fresh sprouted seeds on a cookie sheet and bake for one hour at 100ºC. The maltose will caramelize and give the grain a light brown covering.

Lesson 3 Water Activity – What is it? Thought Experiment

Imagine you were placed in a large plastic cylinder. The floor is not solid but a grating under which a powerful fan is blowing air past you straight up and out the top. Moving all around you, propelled by the force of the 'wind' is a large number of one hundred dollar bills. The top of the cylinder lets the air escape. You notice that the occasional 'bill' escapes but it is quickly replaced with a new one to keep the number of bills in the cylinder constant. As a scientist you cannot help but see the entire system is at equilibrium, an equilibrium you will soon change. In ten seconds you will be free to keep all the hundred dollar bills you can catch and hold in sixty seconds. Buzz! You grab wildly, but, too soon, the time runs out. For now the fan is still blowing. The occasional bill still leaves the top of the cylinder and is replaced by another; a new equilibrium has been reached but this time with some of the money safely, and firmly, locked in your iron grasp.

Compare the above story to water molecules in a container.

Ask students:

Question: What do the 'bills' moving freely within the cylinder represent?
Answer: Water molecules moving through the liquid.

Question: What is represented by the 'bills; that occasionally escape out the top of the cylinder?
Answer: Water molecule that escape the surface of the water into the vapor phase

Question: What is indicated by the fact that every 'bill' that escapes is replaced in the container by another 'bill' from above?
Answer: The system is at equilibrium. There is no change in the number of bills in the container. For every bill that leaves the container, one is replaced immediately.

Question: If the fans power were increased what would happen to the number of escaping bills?
Answer: The number of bills escaping from the top would increase.

Question: If the number of escaping bills exceeds the ability to replace them what will eventually happen?
Answer: The system is not at equilibrium any more. More bills are escaping through the top of the container than can be replaced. Eventually, all the bills will escape and none will remain in the cylinder.

Question: If, as the number of escaping bills increased, the number of replacement bills still equaled the escaping bills, what would have happened?
Answer: The system would still be at equilibrium.

Question: How is this like what happens in water as it is heated?
Answer: As you put heat energy into the water, the molecules become 'more active', with more escaping. By escape we mean that the water molecules transform from the liquid phase into the vapor phase. In other words the vapor pressure over the water increases – due to the increase in the number of water molecules in the vapor phase.

Question: What is this analogous to in water?
Answer: Water boiling.

Question: What was the effect of adding a person to the cylinder before they were allowed to capture any bills?
Answer: Nothing – this would be like putting a rock into a container of water.

Question: What was the effect of allowing that person to 'capture' some of the bills?
Answer: It changed the energy balance in the system. There are a lower number of bills with the required energy (floating free) to escape the top. Fewer bills are floating in the cylinder and escaping the top because the person is holding onto some of the bills. The bills in the persons hand are at a lower energy state as they are 'trapped' or 'bound' by the person. The result is that there are less bills escaping.

Question: How could one describe the two states of 'bills' in the cylinder? (Floating bills and bills held in the person's hand)
Answer: 'Free' or 'active' (bills floating around in the cylinder) and 'bound or 'inactive' (those held in the persons hand) is a suggestion.

Question: How is this analogous to water with salt added?
Answer: Salt dissolved in water changes the energy balance of the system. Some of the water molecules are 'free' floating, while others are 'bound' to the salt ions. There are a fewer number of water molecules with the required energy to escape into the vapor phase. The equilibrium vapor pressure (number of water molecules in the vapor phase) is lowered.

In food or other water containing products, water can exist in both of these states. Some of the water does not interact with the food molecules and can be considered 'free' while the remaining water, which interacts to some degree, can be considered 'bound water'[4]. When molds, fungi or bacteria attempt to grow in food they can access only the free water, not the 'bound' water. To preserve food, think like the person in the cylinder-- if you do not wish to leave a large amount of 'free' money for others, just 'bind' a little extra to yourself.

[4] Note: When water is referred to herein as 'bound water' it should not be thought of as if it were rigidly fixed in place or 'locked' into a matrix or crystal. It may be better to consider the water in an intermediate state, a state between purely free liquid and purely solid—an attached state where the 'bonds' are 'breakable' but not at the energy levels of most bacteria and yeasts.

Lesson 3 Solute and Water Activity Experiment

Background Information

When a solute is added to water, the vapor pressure of the water is decreased. For example, solutions of sugar or salt both have lower vapor pressure than does pure water. This is like the effect of allowing the person to capture some of the bills in the cylinder. The equilibrium vapor pressure was lowered as fewer bills are floating free in the cylinder and thus fewer are able to escape the top.

How much is the vapor pressure lowered? The vapor pressure lowering depends on the concentration of solute particles (or the amount of sugar or salt added to water). If two or more people were allowed to grab the floating bill in the cylinder, then far fewer bills would remain free floating and the amount of escaping bills would be much smaller.

Supplies

- Water
- Salt (NaCl)
- 6 250ml Containers
- Pawkit® Water Activity Meter
- Sample cups (accompanies Pawkit®)

Experiment

Prepare solutions of Salt and Water according to the table below. The students should weigh the water to make an accurate molal solution, but to save time the salt may be added to 100ml of water. Have the students compare the volume of salt/water solutions in each container. This will help illustrate the differences between molal and molar solutions.

After all of the salt is dissolved, have the students measure and record the water activity of each solution. Have the students plot the molal concentration versus water activity on a graph. How is the water activity affected as the concentration of salt increases?

Molal[5] Solution	Distilled Water (kg)	Salt (NaCl) (g)	Water Activity
1	0.1	5.85	
2	0.1	11.7	
3	0.1	17.55	
4	0.1	23.4	
5	0.1	29.25	
6	0.1	35.1	

[5] Note: this is molal; not molar hence each requires ‘n’ moles/kg H_2O.

Water Activity Scavenger Hunt:

How many items can you locate that have water in them? Food ? Cosmetics? Lotions? Test several items and plot each on a graph with the salt solutions. How does each compare? Conclusions?

Answer Key

Molal Solution	Distilled Water (kg)	Salt (NaCl) (g)	Water Activity
1	0.1	5.85	**0.966**
2	0.1	11.7	**0.931**
3	0.1	17.55	**0.893**
4	0.1	23.4	**0.851**
5	0.1	29.25	**0.807**
6	0.1	35.1	**0.760**

Lesson 3 Freezing Point Depression – Making Ice Cream Activity

Background Information

Adding salt to water changes the vapor pressure, boiling point, and freezing point. As illustrated in the previous experiment the amount of change depends on the amount of salt added. The freezing point corresponds to the temperature at which the vapor pressures of the solid and liquid phases are the same. For a salt/water mixture the temperature at which the solution and solid phases will have the same vapor pressure is reduced.

One popular story alleges that Fahrenheit used the lowest melting point he could obtain with a salt/ice mixture as 0° on the temperature scale that bears his name.[6] Regardless of whether that is true, it is true that salt lowers the melting point of ice and we will demonstrate the usefulness of that today by making ice cream without a freezer.

What does the salt do? Just like we use salt on icy roads in the winter, salt mixed with ice in this case also causes the ice to melt. When salt comes into contact with ice, the freezing point of the water is lowered. Water will normally freeze at 32°F. A 10% salt solution freezes at 20°F, and a 20% salt solution freezes at 2°F. By lowering the temperature at which water freezes, we are able to create an environment in which the milk mixture can freeze at a temperature below 32°F into ice cream.

Supplies

Here is the recipe. It is only enough for one student so each student can make his or her own.

- 1/2 cup whole milk or 'half and half'
- 1/2 teaspoon vanilla
- 1 tablespoon sugar
- 4 cups (crushed) ice
- 4 tbsp Rock Salt (see text)
- 2 quart size Zip-loc bags
- 1 gallon size Zip-loc freezer bag
- a hand towel or gloves to keep fingers from freezing as well!

Experiment

Mix the milk, vanilla and sugar together in one of the quart size bags. Remove as much air as possible before sealing the bag tightly. Place the bag inside another quart size bag while leaving as little air inside as possible. Seal well (Note: double-bagging is not

[6] Another legend claims that 0º was simply the coldest temperature measured in the winter 1708-09 in Fahrenheit's hometown of Gdansk (Danzig).Still others claim Fahrenheit simply modified the Romer scale to make it easier to use.

necessary but it does minimize the risk of breakage and of salt and ice getting into the ice cream). Put the quart bag(s) inside a gallon size bag and add enough crushed ice to the bag to cover the quart bag. Sprinkle the salt on top of the ice. Remove most of the air from the large bag before sealing it. Wear gloves or wrap the bag in the towel and shake/ massage the bag, making sure the ice surrounds the cream mixture. It may take as much as ten minutes for the mixture to freeze into ice cream.

Note on rock salt: The amount of salt called for in the recipe is about 4 tablespoons. Let the students experiment by adding more salt, less salt, or even no salt to see how the freeze time is affected. Record the time required to freeze and the amount of salt added to the mixture. Is there an optimum amount of salt?

When the ice cream is finished, carefully remove the inner bag and enjoy the treat. Save the contents of the outer bag. Measure the water activity of each sample and compare the reading to the time required to freeze the treat. Note: you will have to warm the salt water solutions prior to measuring the water activity in the Pawkit water activity meter. How well does coldness of the mixture (freeze time) correlate to water activity?

<u>Explanation</u>

Melting ice requires the addition of heat in the amount of 80cal/g (or stated in other units 3.34 J/kg). If ice is added to water, the melting ice will remove heat from the water. If enough ice is added, the mixture will reach equilibrium at 32°F (0°C). At equilibrium a water molecule is equally likely to leave the ice crystal, as it is to join it.

Dissolving salt into the ice/water system disrupts this state of equilibrium. In solution, the salt separates into sodium and chlorine ions. These ions with their respective electrical charges, attract and 'bind' the molecules of liquid water, leaving fewer water molecules to combine with the ice, while water molecules from the ice continue leaving the ice crystal as easily as before. The net result is more heat energy is required from the system to accommodate the melting ice. Since melting ice requires heat, and the primary heat source is the ice cream mixture, the temperature of the mixture drops as it loses heat to the melting ice.

Lesson 4 The Search for Food Immortality

If you were to choose the perfect storage food, something that could be set aside and still be edible in the distant future what would it be and why?

Allow time for discussion. This purpose of this discussion is not to find the 'one perfect food' but rather to explore the reasons some foods might be preferred over others. Here is a sample list of criteria that could be use to encourage discussion.
How important is:

- taste
- appearance
- nutrient content
- nutrient density
- weight
- serving size
- readily available energy

One well-known food that students may have mentioned as having a long shelf life is the Hostess Twinkie®. Despite urban legends to the contrary, stories of '100 year old' Twinkies® are an obvious exaggeration since the confection was first baked in 1930. Which leaves one to wonder is a 100 year old fruitcake a possibility?

Lesson 4 Student Lab Experiment: Twinkies®

Testable Question: If a Hostess Twinkie® is left undisturbed in the wrapper, will the moisture from the filling migrate to the cake making it 'soggy'?

Hypothesis: If the water activities of the two components are the same, then no moisture will exchange between the two components.

Materials Required:

- Pawkit water activity meter with sample cups
- Hostess Twinkies®
- Oreo®
- Ritz® bits

Observations:
Pass out Twinkies to each lab pair. Ask them to examine the snack food without opening the package.

Question: What can you know about the treat without opening the wrapper?
Observations may include comments nutrition facts, ingredients, color and texture.

Question: What else do we know, or suspect, about the snack food from prior experience?

Carefully open the wrapper and break a cake in two pieces. Examine the cake. What do you notice? Examine the cake and filing. How are they similar, different?

What is your hypothesis and what is it based on?

Procedure:

1. Carefully open the cream filled cake. Remove a small amount of the cream filling. Measure the water activity of the filling using the Pawkit water activity meter. Place a small amount of the cream filling into a sample cup (be sure to cover most of the bottom of the sample cup). Place the cup into the Pawkit water activity meter. Press button 1 once to turn the Pawkit on and a second time to start the water activity measurement. After five minutes the Pawkit will beep 4 times and the final water activity of the filling will be displayed. Record the water activity result in the table.

2. Use a clean dry sample cup (either a new sample cup or carefully clean and dry the sample cup use earlier) to measure the water activity of the cake. Remove a small amount of the cake and place it in the sample cup. Determine its water activity as described above. Record the water activity result in the table.

3. Based on your observation what will be the expected water activity of the finished product (cake and filling together)?

4. Thoroughly mix a small amount the cake and filing. Place this mixture in the sample cup. Determine its water activity as described above.

5. Repeat the above steps with a sandwich cookie such as Oreo® or Ritz® bits

Questions:

How does the a_w (water activity) in the cookie filling compare to the filling in the Twinkie®?

Why do you suspect they are different/similar?

	Water Activity (a_w)		
	Creamy Filling	Cake	Mixture
Twinkie®			
Oreo®			
Ritz® bits			

Lesson 4 Cheese and Cracker Experiment

Another food is the Crackers and Cheese combo marketed with the cheese and crackers in separate, but attached, containers. Ask the students why they suspect one product is marketed as a finished product (Twinkies®) while the other is marketed in separate containers. Is it possible to market cheese and crackers together? What would happen if the cheese and cracker were not packaged separately?

Testable Question: If the Cracker is exposed to the Cheese in a Cheese & Cracker Snack will the moisture migrate and change the texture properties of the cracker (making it 'soggy')?

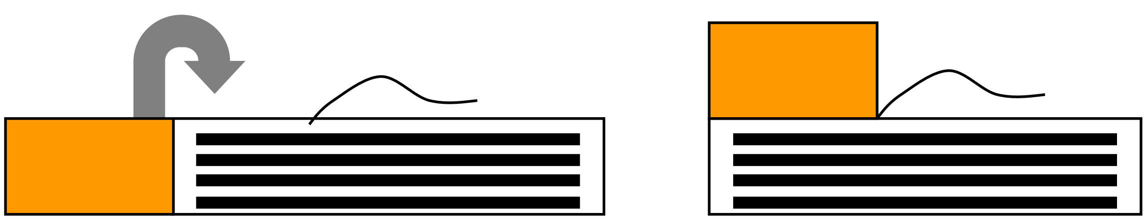
Hypothesis: If the water activities of the two components are the same, then no moisture will exchange between the two components.

Materials Required:

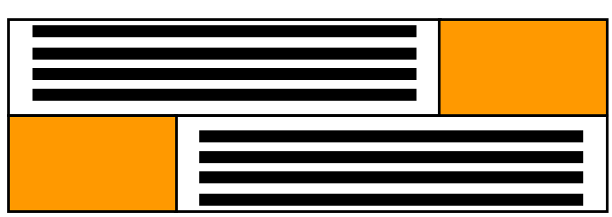
- Pawkit water activity meter with sample cups
- 2 Kraft Cheese N' Cracker Snack Combo

Note: You will need to expose the crackers to the cheese for at least three hours prior to measuring the water activity. The experiment may be prepared on the day prior to measuring the water activity.

Gently peel the cover from one package of cheese and fold it over onto the partially uncovered crackers. When you are sure the seal is airtight set the product aside. The diagram below shows the finished products.



Alternatively, two packages may be place on top of each other and held together with a rubber band.



Alternatively, the opened Cheese N' Crackers package may be sealed in an airtight sandwich bag.

Procedure:

1. Open the intact container and measure the a_w of the cheese and cracker separately. Place a small amount of the cheese into a sample cup (be sure to cover most of the bottom of the sample cup). In a second sample cup break the cracker into small pieces that fit easily into the cup. Place the cup into the Pawkit water activity meter. Press button 1 once to turn the Pawkit on and a second time to start the water activity measurement. After five minutes the Pawkit will beep 4 times and the final water activity will be displayed. Record the water activity result in the table.

2. Now open the altered package and examine the contents. What do you notice about the cheese? What do you notice about the crackers? Why do you suppose this happened?

3. Measure the water activity of the altered cheese and crackers separately. Place a small amount of the cheese into a sample cup (be sure to cover most of the bottom of the sample cup). In a second sample cup break the cracker into small pieces that fit easily into the cup. Place the cup into the Pawkit water activity meter. Press button 1 once to turn the Pawkit on and a second time to start the water activity measurement. After five minutes the Pawkit will beep 4 times and the final water activity will be displayed. Record the water activity result in the table.

	Water Activity (a_w)	
	Cheese	Cracker
Intact package		
Altered package		

4. Why does the food company package the cheese and crackers separately? Based on your observations from the cheese and cracker experiment - what do you think would happen if you used another combination food that is packaged separately?

Question:

There are many ways to prevent the growth of microorganisms - one is limiting a_w. Based solely on your a_w measurements, what types of microorganisms may be inhibited from growing in the cracker? In the cheese?

Lesson 4 Discussion / Thought Experiment

The table below shows individual components from foods that are multi-component - predict what a water activity check will reveal. Space is left for you to make a prediction of your own. When you have made your predictions, test as many as possible. Save small samples of food from home for testing if you can.

Food #1	Food #2	Expectation & Results
Pizza Crust	Pizza Sauce	
Chocolate Éclair -- Marshmallow filling	Chocolate Éclair --cracker	
Cake	Frosting	
Fruit pie crust	Fruit pie filling	
Cereal flakes	Fruit pieces (raisins, etc.)	

Questions:

1. Based on your knowledge of water activity and how it relates to water movement, what will happen to the water in a cream filled chocolate candy? Be certain to identify the candy and give reasons for your predictions.

2. Consider the peanut butter and jelly sandwich. In a normal peanut butter and jelly sandwich there is a slice of bread, a layer of peanut butter, a layer of jelly, and then a second slice of bread. What happens to this sandwich as it is stored?

3. Now consider a different type of peanut butter and jelly sandwich:
Bread – Peanut Butter – Jelly – Peanut Butter – Bread
What do you predict would happen to this sandwich during storage? Would the bread become soggy over time?

4. Now omit the peanut butter entirely leaving a jelly sandwich. What purpose does the peanut butter serve besides adding flavor?

Lesson 5 Browning – Sugar's Dark Side?

Browning is of great importance in food and its results can be either desirable or undesirable. For example, the brown crust formation on bread is desirable; the brown discoloration of dry milk powder is undesirable. For products in which the browning reaction is favorable, the resulting color and flavor characteristics are generally experienced as pleasant. In other products, color and flavor may become quite unpleasant.

Browning of foods is due to enzymatic or non-enzymatic reactions. Enzymatic browning or oxidative, is a reaction between oxygen and a phenolic substrate catalyzed by polyphenol oxidase. This is the common browning that occurs in cut apples, bananas, pears, and even lettuce, and it does not involve carbohydrates. Non-enzymatic browning involves the phenomena of caramelization and/or the interaction of proteins or amines with carbohydrates. The latter reaction is the Maillard reaction.

Lesson 5 Browning of apple slices

Testable Questions:

What happens to cut fruit that is left to sit in the air?

Why does food turn brown?

Does all food turn brown?

Is 'browning ever desirable? (Consider the browning of meat and bread {toast})

What can be done to prevent food from turning brown?

Hypothesis: The browning of apples slices can be controlled by dipping the slices into different solutions.

Materials Required:

- Apples
- Knife
- 8 Small bowls or cups
- Substances to cover the apple slices
  - Corn syrup or sugar water
  - Lemon-Lime soda
  - Salt
  - Baking soda
  - Lemon (or Lime) Juice
  - Vitamin C tablet dissolved in water
  - Apple Juice
  - Cider Vinegar
  - Cellophane wrap (to exclude air as a factor)

Note: These are suggested materials to dip the apple slices. Feel free to substitute.

Procedure:

1. Prepare solutions of the different substances to dip the apple slices in the bowls or cups.

2. Slice an apple[7] into thin slices.

3. Set one slice aside as the control.

4. Dip the other slices of apple into various liquids then set each aside to observe the 'browning' reaction.

5. Cover one slice in clear cellophane wrap to exclude air as a factor.

6. When the control apple slice is 'brown' rate the comparative 'browning' of the other slices. Record your results in a table.

Substance With Which The Apple Slice Was Covered	Observed Result
Control	
Corn Syrup or Sugar water	
Lemon-Lime Soda	
Salt water	
Baking soda in water	
Lemon (or Lime) Juice	
Vitamin C tablet dissolved in water	
Apple Juice	
Cider Vinegar	
Cellophane wrap	

[7] Be sure to use an apple that browns easily. Some cultivars such as the Cortland brown little or not at all and, while they are suitable for eating or baking, they are not suitable for this demonstration.

Discussion:

Why did some materials prevent browning while other did not?

How could you test your hypothesis?

Many people add a small amount of lemon juice to fruit when they are baking. Why might this be helpful?

Based on your observations what else might be as, or more effective, than lemon juice?

Citric Acid or Ascorbic Acid (Vitamin C) are sometimes added to commercially prepared foods to prevent browning. Why?

Lesson 5 Browning of bread

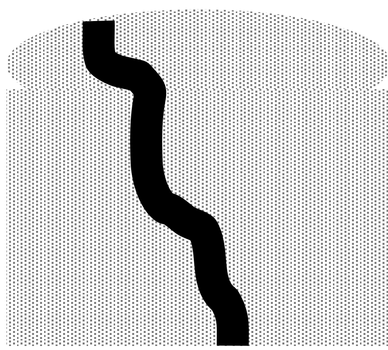
Testable Question: If lemon juice can prevent browning of fruit will it also prevent browning of bread?

Supplies:

- Bread
- Lemon juice
- Toaster oven
- Pawkit water activity meter
- Toothpicks (or similar devices)
- Paper

Procedure:

1. Pour a small amount of lemon juice on a slice of bread being careful to only get the lemon juice on a small potion of the bread. The idea is to determine if the presence of the lemon juice inhibits toasting so most of the bread should remain untouched. The illustration below show how this could look.



Bread slice with small streak of lemon juice.

2. Place the bread with small streak of lemon juice into a toaster and toast. What happened? Did the lemon juice area of the bread toast similarly to the rest of the bread?

3. Measure the water activity of the 'lemon juiced' area, the toasted area and compare to a control (un-toasted) sample of bread.

Question: Based on your observations what are two possible reasons the bread did not toast?

Hypothesis 1: The acid (lemon juice) prevented browning

Hypothesis 2: The bread is too wet from the lemon juice

Test for Hypothesis 1
An old method of creating 'invisible ink' is to use lemon juice. You will repeat this process while testing the 'acid hypothesis'.

1. Dip a toothpick or similar device into lemon juice and write a message on a piece of paper.
2. Allow the paper to dry naturally -- do not hurry the process with heat. When the lemon juice is dry the message will be 'invisible'.
3. Gently heat the paper. The lemon juice will 'brown' exposing the message.

Clearly, lemon juice is capable of browning so it appears that water content (Hypothesis 2) was responsible for the lack of browning of the bread. How could you test this hypothesis? Construct an experiment and test it.
(Here is a hint: substitute pure water for the lemon juice and repeat the above procedure.)

Clearly some water must be removed for toasting bread. Could other means to remove water also toast bread?

Test for Hypothesis 2 - How Not To Toast Bread
- Place a piece of bread in the microwave oven. You may wish to wrap the bread in a paper towel to absorb any water that is released.
- Microwave on high for 20 to 40 seconds. Do not microwave too long or you will burn the bread producing a foul odor.
- Allow the bread to cool before you remove it from the microwave. Tear the bread in half. If you have done this correctly the bread will be partially toasted. How do the results compare with a conventional toaster?
- If you have access to a conventional oven, place a piece of bread in the oven and bake it at a temperature of about 200ºF (93ºC) for thirty minutes. Remove the bread and examine the result. How does this method of toasting compare with the other two?
- Check the water activity of both pieces of toast. Are you able to make bread too dry to toast?

> To the instructor: If done properly the microwave toasted bread will be 'toasted' on the inside only whereas the oven baked bread will be toasted throughout.

Discussion Question: If the acid in lemon juice prevents browning in apples why does it not affect bread the same way?

Answer: It would seem obvious the browning mechanism must differ between bread and apples. In fact apple browning is an enzymatic reaction involving oxidation. Enzymatic browning is generally considered beneficial in coffee and chocolate while no so in apple and potatoes. Most enzymatic browning occurs at water activity levels above 0.8 so lowering water activity, eliminating oxygen, or sufficiently lowering the pH (by adding lemon juice) will inhibit most enzymatic browning reaction.

In addition to enzymatic browning some foods react in a way called non-enzymatic browning. The brown color of roast meat or the familiar brown color of caramels are two examples and represent the two types of non-enzymatic browning: Maillard browning and caramelization. Maillard browning is the reaction of a sugar and an amine and is accelerated at cooking temperatures. Caramelization, sometimes referred to as thermal decomposition, requires low water and high sugar content and occurs at medium to high[8] temperatures. However, low pH does not inhibit non-enzymatic browning, which is why the lemon juice prevents the browning of apples (enzymatic browning) but still browns at higher temperatures.

Since non-enzymatic browning requires sugar, it is possible to determine which type of browning is responsible for the brown color in cookies. Simply replace the sugar with a sugar substitute, such as Sugar twin®. According to the Sugar twin® website http://www.sugartwin.com/bt.cfm, Sugar twin® does not 'brown' in baking. They recommend to bakers that they add a small amount of sugar (*Sounds like grounds for an edible experiment?)* if browning is desired

Non-enzymatic browning requires the presence of sugar. If you compare recipes for meat bastes you will find they often list corn syrup as one of the ingredients. Corn syrup contains a large amount of sugar (reference the label). Why might corn syrup be a major ingredient is meat bastes?

Hint: think about the Maillard (protein + sugar) reaction in browning and the new flavors that are created.

For more information about browning in food please visit the following website: http://www.wateractivity.org/theory.html

[8] Fructose 110ºC, Glucose & Sucrose 160ºC, Maltose 180ºC

Lesson 5 Supplement: Making your own pH Indicator

Testing the various materials in which the apple slices were dipped can reveal their approximate pH. Testing pH is one way for student to see if basic or acidic solutions can prevent enzymatic browning. Red cabbage is a commonly available source of a pH indicator solution. Red cabbage contains an anthocyanin[9] pigment molecule that will turn a red color in highly acidic solutions and greenish yellow in strongly basic solutions. Neutral solutions result in a purplish color.

Therefore, it is possible to determine the pH of a solution based on the color it turns the anthocyanin pigments in red cabbage juice. The color of the juice changes in response to changes in its hydrogen ion concentration.

$$pH = -\log[H^+]$$

Acids donate hydrogen ions in an aqueous solution and have a low pH (pH<7). A neutral solution has a pH = 7. Bases accept hydrogen ions and have a high pH (pH>7).

Below is a table showing the approximate colors red cabbage solution will indicate at different values of pH.

pH	2	4	6	8	10	12
Color	Red	Purple	Violet	Blue	Blue-Green	Greenish Yellow

Supplies:
- Red Cabbage
- Beaker or pan
- Hot plate or stove
- Filter paper (coffee filters work well)

Procedure:
1. Chop, tear, shred or grate the cabbage into small pieces until you have about 2 cups of 'chopped' cabbage. Place the cabbage in a stainless steel pan or large glass beaker. Cover with water and boil gently for several minutes to allow the color to leach out of the cabbage. If you do not wish to boil the cabbage simply let it soak in the water a few hours to overnight.

---

9 Anthocyanin (from the Greek anthos = Flower, kyáneos = purple) is a water-soluble pigment responsible for the reddish to bluish hues found in berries, fruits and leaves. Water plants do not produce anthocyanin but most land based plants do, cactus (which instead contains betacyanins) are one common exception. Anthocyanin is a powerful antioxidant that also strongly absorbs UV light simultaneously protecting the plant and attracting insects. Relatively high concentrations of anthocyanins are found in: chokecherry, cherry, purple grapes, blueberries and, of course, red cabbage.

2. Allow the liquid to cool. Pour only the liquid into a glass container while leaving the plant material behind. The indicator solution (water) may be violet to blue depending on the pH of the water.

3. Divide the indicator solution among several small glass containers. Add a strong acid to one and notice the color change. Add a small amount of a strong base and observe the change in color.
Some common household acids and bases are given below.

4. Now test each substance that was applied to the apple in the browning test and compare the results. What do you notice?

<u>Alternate Method</u>: This will require some advance preparation. Place a clean coffee filter into a ***concentrated*** solution of red cabbage indicator. Allow the filter to soak for several hours or overnight. Remove the filter and hang it up to dry. You may now cut the filter into thin strips and use them to test pH.

Note:

- This demo uses acids and bases, so please make certain to use safety goggles and gloves, particularly when handling strong acids (HCl) and strong bases (NaOH or KOH).
- Chemicals used in this demo may be safely washed down the drain with water.
- Use separate containers for each household solution – you **do not** want to mix chemicals that do not go well together.
- Household acids and bases:
  - Household ammonia (NH_3)
  - Baking soda (sodium bicarbonate, $NaHCO_3$)
  - Washing soda (sodium carbonate, $NaCO_3$)
  - Lemon juice (citric acid, $C_6H_8O_7$)
  - Vinegar (acetic acid, CH_3COOH)
  - Cream of tartar (potassium bitartrate, $KHC_4H_4O_6$)
  - Antacids (calcium carbonate, calcium hydroxide, magnesium hydroxide)
  - Seltzer water (carbonic acid, H_2CO_3)
  - Muriatic acid, masonry's cleaner, pool acid (hydrochloric acid, HCl)
  - Lye (potassium hydroxide, KOH or sodium hydroxide, NaOH)

Lesson 6 Grain & Seed Preservation

Acme Premium Seed Company[10] has harvested next season's seed from the fields and wishes to store it in a way that will maximize germination.

Safe-Store Grain Elevators buys grain from local farmers and sells it around the world. The grain must be stored in a way that ensures the grain will be as wholesome and safe as when it was purchased from the farmer.

Medieval Farms is a modern-day reconstruction of a European medieval village. They grow wheat, barley, and rye using traditional methods. They store their own grain but use modern science to ensure there are no repeats of the ergot fungus episodes that trouble medieval Europe.

Eco-Housing for the World builds low cost housing in developing countries using straw bales. The straw bale walls are covered with a semi-permeable material to keep the rain out but must be properly dry to ensure mold and mildew cannot grow within the walls

Biggy-Pop Popcorn wants to make sure their premium popcorn makes the largest and fluffiest popcorn. To do so they carefully monitor the storage conditions to ensure uniform results.

Old-Tyme Seed Inc. sells 'heritage' seed to home gardeners. These seeds typically represent now rare, non-commercial varieties grown to preserve 'a taste of the past'. The seeds contain unique and irreplaceable genetic material so extreme care is taken in storage and shipping to preserve viability.

Question: What do all these 'companies' have in common?

Suggestion for discussion -- They all must be concerned with achieving suitable levels of active water in their product?

[10] The names of companies in these scenarios are, of course, entirely fictional. Any resemblance between these names and actual companies is entirely coincidental.

Lesson 6 How Does Water Activity Affect Sprouting Rate?

Testable Question: How much water is optimum for storing seeds?

Hypothesis: Drying seeds will allow them to be stored longer, but if they become to dry they will no longer be viable

Materials Required:

- dried seeds (beans, lentils etc.)
- coffee filters
- airtight containers such as sealable plastic bags
- Pawkit water activity meter
- Oven at 40°C (110°F)

Procedure:

1. Separate the seeds into five groups of equal number. Set one group aside as the control group.
2. Allow two groups to soak up water by immersing the seeds in water. Remove one group of seeds from the water after 10 minutes and the second group after 20 minutes. Place the seeds on a paper towel to remove excess water. After the excess water is removed, place the seeds inside an airtight bag.
3. Place the last two groups of seeds in a warm oven (about 40°C). Remove one group of seed after 24 hours and the final group after 48 hours. Allow the seeds to cool to room temperature before placing them in an airtight bag.
4. Measure and record the water activity of the five groups of seeds. Place a small amount of the seeds into a sample cup (be sure to cover most of the bottom of the sample cup). Place the cup into the Pawkit water activity meter. Press button 1 once to turn the Pawkit on and a second time to start the water activity measurement. After five minutes the Pawkit will beep 4 times and the final water activity will be displayed. Record the water activity result in the table.
5. Observe the seeds in the sealed bags daily for signs of mold, sprouting, or gas which would indicate the seeds are unsuitable for storage. Record your observations.
6. After a week (or other predetermined time) soak each group of seeds separately overnight.
7. Remove the seeds and place them between two filter papers. Keep the filter paper damp but not wet. When the seeds have begun sprouting measure the germination rate. How does the germination rate compare with the water activity? Is there an optimum level for storage?

	Seed Treatment				
	Control	**Soak 10min**	**Soak 20min**	**Heat 24hours**	**Heat 48hours**
Water Activity					
Seed Storage Observations:					
Day 1					
Day 2					
Day 3					
Day 4					
Day 5					
Germination Rate (after 24 hour soak in water)					
Day 1					
Day 2					
Day 3					
Day 4					
Day 5					

Lesson 6 How Does Water Activity Affect Popcorn?

Testable Question: How does the water activity of popcorn affect the 'popping' rate and the popped kernel size?

Hypothesis: Since 'free' water is necessary for popcorn to pop, popcorn with a higher water activity (more free water) will make larger popped kernels.

Materials Required:

- 1 ½ cups Popcorn
- Popcorn Popper
- Pawkit water activity meter
- Oven at 95ºC (203ºF)
- Glass jar with lid

Procedure:

1. Collect enough popcorn for three batches (about 1 ½ cups). Separate into three equal piles. Set one batch aside as the control group.
2. Place one batch of popcorn into a preheated 95ºC (203ºF) oven for two hours.
3. Place the third batch into a glass jar. Add 1 tbsp. (15ml) water. Put the lid on the jar and shake the mixture to wet the surface of the popcorn. Set the jar aside for about 24 hours. Shake the jar and contents every few hours if you can. If you cannot just do the best you can. It is not necessary to wake up during the night to shake the jar.
4. On the following day measure and record the water activity of each batch, then pop the 'corn' as usual.
5. How do the results compare with your hypothesis?
6. Enjoy the popcorn.

For further investigation – are the results the same for different brands? How about microwave v. non-microwave popcorn?

> Note to the teacher: Popcorn growers and packers have indeed found that too much or too little water affects the size and quality of popped corn. Therefore, they try to pick and package their corn at the optimum moistness for popping. However, after sitting on store shelf or in the consumer home, the product may not be at optimum, hence you may get better results from 'dryer' or 'moister' popcorn.
>
> When popcorn is heated the water inside reaches a temperature of approximately 175ºC and a pressure of about 135 psi before the seed coat ruptures and the internal starch expands greatly and rapidly into popcorn as it cools.

Lesson 6 Grain Storage and Mold Growth

Historical and Modern Significance

Ask the students if they have heard of the ergot fungus. Perhaps some will have heard of the incidences of ergot poisoning of grain in medieval France. There were many incidences of ergotism[11] from eating tainted grain. The cause was unknown for centuries; since the dark purple ergot sclerotium that replaces the rye grains was believed, until the 1850's to be a part of the plant. Today[12], there is no reason for ergot or the conditions that favor it to be tolerated.

Ergot fungi flourished in rye when the grain was too damp. Today's growers often harvest and buyers often purchase grain based on its tested 'dryness' or water content. Since water content is not a reliable predictor of pathogen growth the grain may be 'extra dry' to provide the conditions that experience has shown are safe. However, since grain is sold by weight, it is in the seller's interest to sell 'wetter' grain. (Remember water content is by weight so even 1% difference can mean a ton of water per 100 tons of grain.)

Is it possible to sell wetter grain that is still safe?

What other property may be used to determine whether grain is safe to store?

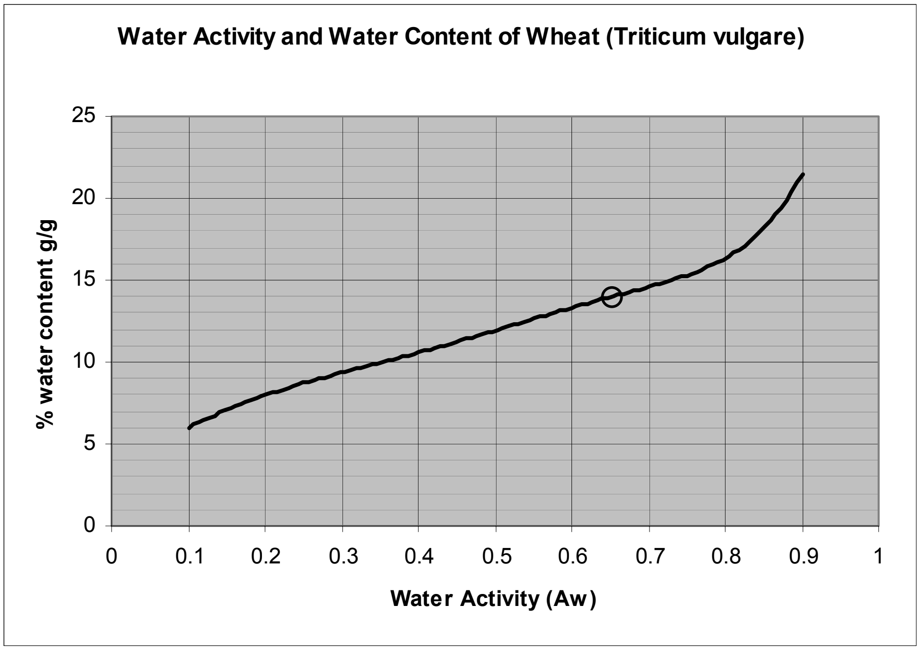
If grain is coated with a chemical or fungicide, how can the true water content be determined? Wouldn't any readings be affected by the presence of the alien additive?

The chart below shows the water content and water activity of wheat at a certain temperature. This relationship is called the moisture sorption isotherm. Maintaining constant water content but varying temperature will cause the isotherm (line) to move horizontally. Maintaining constant water activity but varying temperature will cause the isotherm (line) to move vertically.

Make enough copies of the handout below for every student. A bland 'master' appears at the end of this lesson.

[11] Symptoms included twisting and contorting of the subject's body in pain, trembling and shaking, twisting of the neck and, in some cases, muscle spasms, confusions, delusions and hallucinations. Another form of ergotism, gangrenous ergotism, restricts blood flow to the extremities often resulting in the loss of fingers and toes or arms and legs and sometimes even death. The first know episode occurred in 857 AD in the Rhine valley where the condition was called 'holy fire', presumably because of the burning sensation in the limbs of its victims. Later, it became known as St. Anthony's fire in memory of the monks of the Order of St. Anthony who cared for many of its victims.

[12] The most recent outbreak was in 1951 Pont-St. Esprit, in Provence, France. Infected rye was mixed with wheat before grinding. The resulting bread, when eaten, led to 200 cases of poisoning, four deaths and 32 cases of insanity, including one eleven year old boy who attempted to strangle his own mother. Today rotation cropping and washing seed in a 30% KCl solution to separate the ergot from the seed has all but eliminated ergot as a problem.

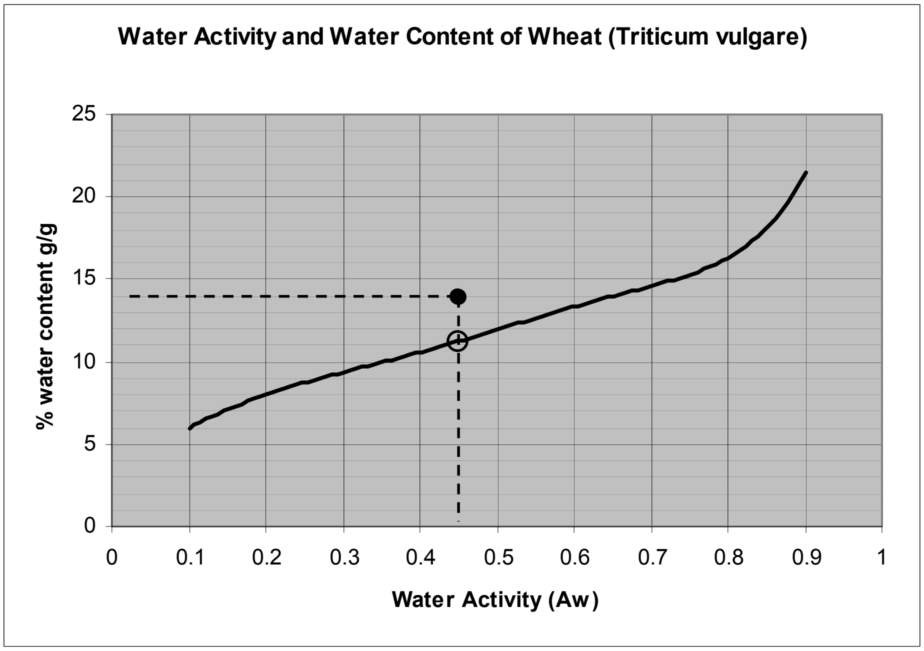


If wheat is sold at 14% moisture (at the temperature of the isotherm) what is the relative water activity? Will the grain mold?

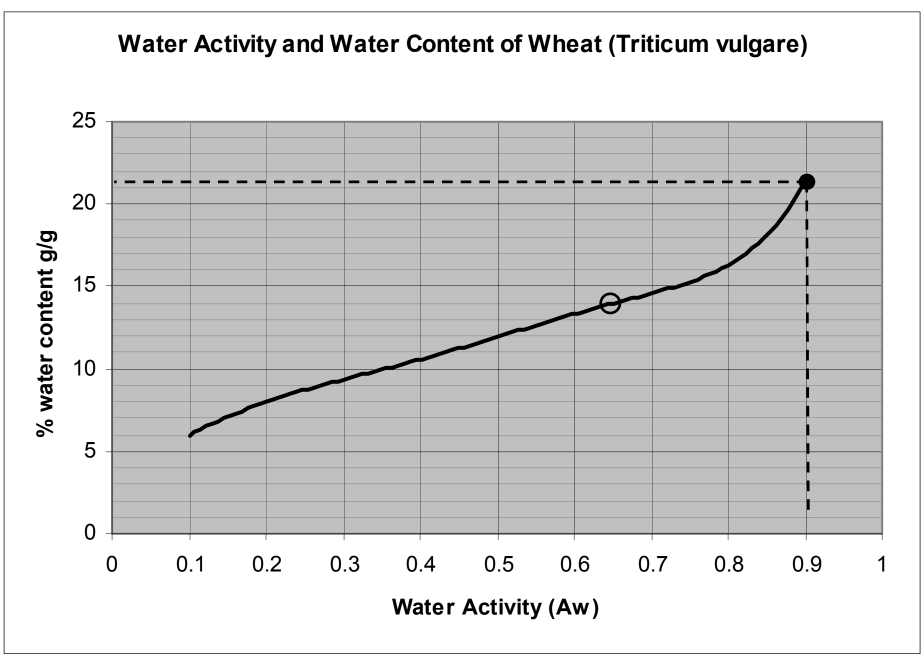
About 0.65, No, most mold will not grow below 0.70 and even 'dry-loving' Xerophilic molds will not grow below 0.65.

If the grain was harvested at 14% water content but was stored at 45% relative humidity, will the grain absorb water?

Refer to the diagram below. The 45% humidity (water activity) line does not intersect with the 14% water content on the isotherm. Clearly equilibrium cannot be reached until either the humidity or the water content changes. If the container is sealed, water will leave the wheat until equilibrium is reached. If the container is adequately vented, water will continue to leave the wheat until it reaches about 11% moisture (0.45 a_w). In other words, if the humidity is lower than the water activity the grain will 'dry' until equilibrium is reached. If the humidity is greater than the water activity the grain will gain moisture until equilibrium is reached. Measuring water activity directly avoids the need to find and use a product and temperature specific isotherm.



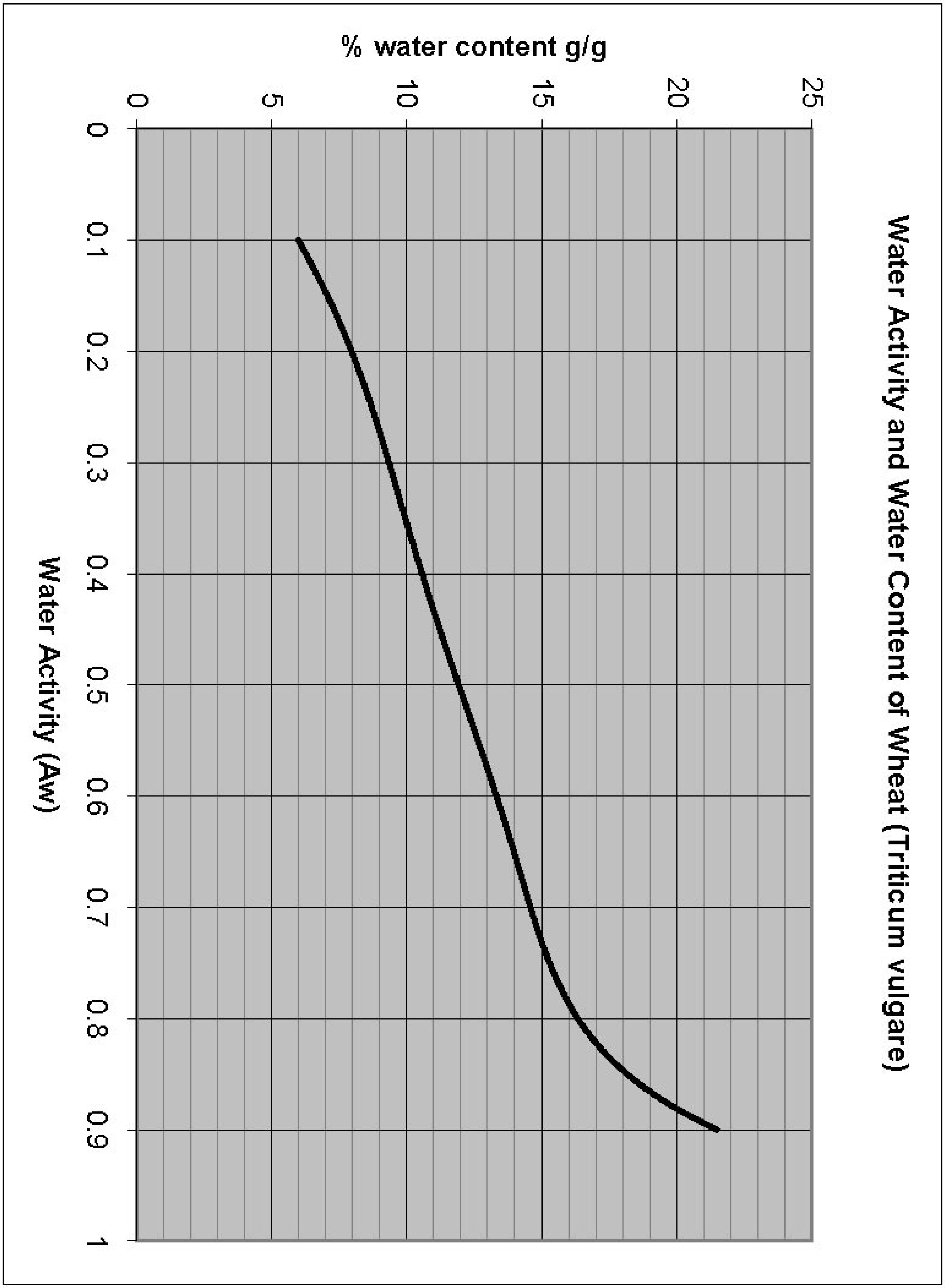
Problem: Safe-Store Grain Elevators wishes to ship 100 tons of grain on a barge. During transit it is estimated the grain will be exposed to humidity of up to 90%. If the estimates are correct, what could be the potential water content of the exposed grain? Is the grain susceptible to mold? If the barge is filled to the water line will it float higher or lower in the water as it reaches its destination?



Writing/Research topic: Water activity has been demonstrated to predict whether microorganisms will be inhibited. Imagine you had a time machine and could travel to any time in the past and prevent the outbreak of a serious food-borne illness where would you travel to?

Note: Some historians suggest that food-borne pathogens may have contributed to: the division of the abdication of Charles III and the break-up of Holy Roman Empire, witchcraft hysteria and trials, and the extraordinary high death rate of the 'Black Plague' around 1350. What do you think?

Note: The information on the following chart is accurate, but is intended for educational purposes only


Water Activity and Water Content of Wheat (Triticum vulgare)
% water content g/g
0
5
10
15
20
25
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Water Activity (Aw)

Lesson 7 Pharmaceutical Applications of Water Activity

Most over-the-counter pharmaceuticals and cosmetics include water in their recipe. Water may be just an inert ingredient or it may be more. How do you know when you have just enough?

Testable Question: Today we are going to begin a series of experiments to test whether there is an optimum quantity of 'free' water in two personal care products: shampoo and skin cream. When we are finished you will have data showing which shampoo and skin creams your classmates find superior.

Supplies:

- Pawkit water activity meter
- Small containers (e.g. 35mm film canisters)
- 3x5 index cards
- envelopes

Procedure:
Note: This procedure requires more than one day to complete. Pre-number the containers, index cards, envelopes.

Students should be allowed to 'opt out' of this experience if they feel uncomfortable. If a student has known skin sensitivity they, of course, should not apply unknown substances to their skin.

1. Ask all students to pick up an empty container, a 3x5 index card, and an envelope to seal the index card within. At home, the students will fill the container with enough shampoo for one use plus enough to determine its water activity (a_w). (Ideally the shampoo will be collected in identical containers such as 35mm film canisters.) The students will record their name and the brand of shampoo on the 3x5 index card and place the card into the envelope. Samples will be identified by the number on the container, index card, and envelope.

2. Measure and record the water activity values for the different shampoo samples on the 3x5 index cards. Place a small amount of the shampoo into a sample cup (be sure to cover most of the bottom of the sample cup). Place the cup into the Pawkit water activity meter. Press button 1 once to turn the Pawkit on and a second time to start the water activity measurement. After five minutes the Pawkit will beep 4 times and the final water activity will be displayed. Note: since the water activity test is non-destructive the shampoo sample may be returned to the container to ensure there is enough sample for the following tests. The water activity value may be written on the index card or in a table with the corresponding sample number.

3. Randomly assign the samples to other students. Record the name of the student who will take the shampoo home for use on the 3x5 index card. Neither the users nor evaluators will know the name of the product until after final evaluations are complete. This is a double blind study.

4.

You may wish to use the next several minutes to create a worksheet to evaluate the shampoo. What is most important cleanliness? 'Silky, shiny' hair? A well conditioned look? If you are having trouble getting started maybe the form below will help.

Shampoo number ________

On a scale of 1 to 5 rate the following characteristics

The hair looks clean but not stripped of natural oils

1...........2...........3.............4.............5

The hair looks soft, silky, and manageable

1...........2...........3.............4.............5

I would use this shampoo for my own hair

1...........2...........3.............4.............5

Whatever form is created make enough copies for each student to evaluate several of his/her classmates. Each shampoo should have at least 10 evaluations if possible.

As late as is reasonably possible, but before the next class, you will wash your hair with the 'mystery shampoo'. When you return to class the effects of the shampoo on your hair will be rated. The raw score data will be compiled and when it is summarized the brand names will be revealed.

5. When students return the following day - have half of the class sit so that the other half can evaluate the effects of the shampoo. Have the other half complete the rating forms and compile them by shampoo number. When the first half has finished have the students change places and repeat the procedure. You may need to remind the students that <u>judging is done by observation and does not include physical contact of any sort</u>.

6. When all data is collected give each pair of students two sets of shampoo to evaluate. Ask them to compute the average score for each category and the total average score.

Have the students complete the following data sheet for each shampoo as the data is reported. From the 3x5 card completed earlier provide the water activity (a_w) for each sample. If computers and a spreadsheet program are available ask the student to enter the data and sort by average score and water activity. Do the data pairs correlate? If so how? Negatively? Positively? Does it appear that the data correlates in a significant way? When the students have finished processing the data ask them to print the results. Discuss the findings. Finally, reveal the names of the shampoo products?

Lesson 7 Hand Cream Test

This is similar to the shampoo test. As with shampoo test students should be allowed to 'opt out' if they feel uncomfortable. If a student has known or suspected skin sensitivity they of course should not participate and should not apply unknown substances to their skin.

- Have the students provide unlabeled samples from a variety of skin/hand/face/moisturizing cream.
- Create a worksheet to evaluate the skin cream. An example is provided but students should be encouraged to produce their own judging criteria.
- Ask each student to apply three types of skin cream and rate each. Ask the rating to be repeated after several minutes.
- Collate and evaluate the results as before.
- Reveal the brand name only after the data analysis is complete.
- Facilitate a class discussion on the results.

Note: One possibility to speed the process is to divide the class in half and have half do each test. Groups may be selected randomly or you may wish to have boys use the shampoo and girls test the skin cream (or vice versa).

Name: ________________________________ Period: ________

Experimental Design Planning Sheet

The **investigative (testable) question** I am trying to answer is	
The **hypothesis (prediction)** *(related to the investigative question)* I am making is …	I am making this prediction because…
Materials/Tools/Technology (LIST) (Equipment used in this investigation)	**Procedure and Safety Requirements:** (List of steps, including safety requirements, to be performed when I actually DO the investigation -- repeatable by someone else)
Manipulated (Independent) Variable (What I will change)	
Responding (Dependent) Variable (What I will observe)	
Controlled Variables (What I will keep constant)	
Experimental Control (A standard used for comparison)	

Name: ________________________________ Period: ________

The **chart(s) graph(s)** and/or **data table(s)** we will use to collect and display our data will be shown of additional sheets of paper. The chart(s) graph(s) and/or data table(s) should include the manipulated variable (independent) and responding (dependent) variable, appropriate units and opportunities for **repeated trials**

Conclusion: This is what the data are telling us about how the manipulated variable affected the responding variable.

- Summarize the data and include
  - Manipulated Variable
  - Responding Variable
  - Appropriate units
- Answer the investigative question
  - Include **supporting data**
  - A single data point includes both the manipulated and responding variable
  - Data may include the high and low data points, an average, or a trend analysis
- Explain how the data supports the conclusion:
  - What generalizations can be made from the data

Identify another question that could be answered with a follow up investigation (you may wish to identify possible sources of error in this investigation to justify, in part, further investigation)