Lab 9: Food Preservation by Means of Moisture Control

A relationship exists between the water content of foods and food spoilage, which has been known for centuries by cultures that dehydrate food as a means of preservation. However, absolute water content is not a reliable indicator of food spoilage potential. Water activity is a better indicator and controlling the water activity of a food is a useful method of food preservation. Water activity can influence the rates of microbial growth, enzyme activity, oxidation, vitamin loss, and non-enzymatic browning, although these are not all affected in the same manner.

In terms of microorganisms, water activity can be thought of as a measure of the amount of water available for their growth. The ability of microorganisms to grow is related to the water activity (a_w) of the growth medium rather than to the percentage of water in that medium. Foods with higher water activity contain water that is loosely bound to the other constituents of the system. Foods with lower water activity do not necessarily contain less water, but the water that they contain is more tightly bound and thus not available to support microbial growth. Lowering the water activity of a food, or limiting the moisture available to microbes, will affect their growth and survival rates.

Foods with the same a_w do not necessarily have the same water content. The following systems have the same a_w, even though their total moisture levels differ:

Food system			a_w	%H_2O (dry wt.)
100 g sucrose	+	49 g H_2O	.85	49%
100 g NaCl	+	423 g H_2O	.85	423%
100 g dry fat	+	28 g H_2O	.85	28%

Water activity is defined as the ratio of the water vapor pressure of the system (p) to the vapor pressure of pure water (p_o) **at the same temperature**.

$a_w = (p/p_o)_T$ where $0 < a_w < 1$

For example, the vapor pressure of pure water measured at 25 ºC is 23.80 mm Hg. The water vapor pressure above a saturated salt solution of NaCl is 17.85 mm Hg at 25 ºC. The a_w of this saturated salt solution is:

$$p/p_o = \frac{17.85 \text{ mm Hg}}{23.80 \text{ mm Hg}} = .75$$

The **equilibrium** relative humidity (ERH) of the atmosphere above the solution can be calculated by multiplying the a_w by 100:

$\text{ERH} = a_w \times 100 = .75 \times 100 = 75\%$

If a solution, or food system, and its environment (surrounding atmosphere) are at uniform temperature, and in equilibrium with each other, then the same relationship applies, and a_w can be calculated as follows:

a_w = ERH / 100, where 0 < %RH < 100

A food system will gain moisture from the surroundings if its water activity (x 100) is less than the relative humidity of the environment, and will lose moisture if its water activity (x 100) is greater than the relative humidity of the environment. All other things being equal, this will occur until equilibrium is reached. As an extreme example, imagine a piece of bread in a tent in the Amazonian rain forest and then imagine the same piece of bread in the same tent in the middle of the Sahara Desert. The bread in the rain forest would gain moisture from the surroundings and would probably spoil due to mold growth, while the bread in the desert would lose moisture to the surroundings and become inedible due to severe staling. Thus, if no protective measures are taken, a_w and moisture content will be influenced by the ERH of the environment.

The relationship between water content and water activity is called a sorption isotherm. In many ways it is analogous to the pH titration curves you have already come across. Both pH and titratable acidity are descriptions of the system, each of which carries consequences. The analogous measure to pH is a_w, and the analogous measure to titratable acidity is water content. Just as knowing the relationship between pH and titratable acidity gives insights into behavior, knowing the relationship between a_w and water content gives insights.

The a_w of a system can be depressed by the addition of solutes as seen by Raoult's Law for ideal solutions:

$$a_w = \frac{n_1}{n_1 + n_2} \qquad n_1 = \text{moles water} \ \& \ n_2 = \text{moles solute}$$

The addition of solutes binds some of the water, making less of it available for microbial growth and chemical reactions. The extent to which the system's a_w is depressed depends on the type and amount of solute added as well as the temperature of the system.

Table 9-1: Equilibrium Relative Humidity's of 15 Selected Saturated Salt Solutions at 10 to 30 ºC.

SALT	at 10 ºC	15 ºC	20 ºC	25 ºC	30 ºC
Lithium bromide	7.1	6.9	6.6	6.4	6.2
Sodium hydroxide	-	9.6	8.9	8.2	7.6
Lithium chloride	11.3	11.3	11.3	11.3	11.3
Potassium acetate	23.5	23.5	23.0	22.5	22.0
Magnesium chloride	33.5	33.0	33.0	33.0	32.5
Potassium carbonate	44.0	43.5	43.0	43.0	43.0
Sodium bromide	60.0	59.0	58.0	57.5	56.5
Cupric chloride	68.0	68.0	68.0	67.5	67.0
Potassium iodide	72.0	71.0	70.0	69.0	68.0
Sodium chloride	76.0	75.5	75.5	75.5	75.0
Ammonium sulfate	81.0	80.5	80.5	80.0	80.0
Potassium chloride	87.0	86.0	85.0	84.5	84.0
Sodium benzoate	88.0	88.0	88.0	88.0	88.0
Potassium nitrate	95.5	95.0	94.0	93.0	92.0
Potassium sulfate	98.0	98.0	97.5	97.0	97.0

Adjustment of a_w

The a_w of a food may be adjusted by the addition of humectants (solutes), freezing, or dehydration. Humectants such as salt (NaCl) or sugar (sucrose) chemically bind food moisture making it unavailable to microorganisms. The effectiveness of ionic vs. nonionic humectants varies in terms of their ability to lower a_w. Given equal molal solutions of NaCl (ionic) and sucrose (nonionic) the NaCl solution will have the lower a_w because it has the ability to bind water better than sucrose. Freezing, which makes water unavailable by physically immobilizing it, may also lower water activity. The a_w decreases as the freezing temperature is lowered. The major methods used to dehydrate or physically remove water from foods are spray-drying, hot-air drying, open-air drying, and freeze-drying.

Table 9-2: Approximate a_w Values of Some Foods and of Sodium Chloride and Sucrose Solutions.

a_w	NaCl (%)	Sucrose (%)	Foods
1.00 - 0.95	0 - 8	0 - 44	Fresh meat, fruit, vegetables, canned fruit in syrup, canned vegetables in brine, frankfurters, liver sausage, margarine, butter, low-salt bacon, bread crumbs.
0.95 - 0.90	8 - 14	44 - 59	Processed cheese, bakery goods, high-moisture prunes, raw ham, dry sausage, high-salt bacon, orange juice concentrate.
0.90 - 0.80	14 - 19	59 - saturation	Aged cheddar cheese, jams, sweetened condensed milk, Hungarian salami, candied peel, margarine, fruit cake
0.80 - 0.70	19 - saturation ($0.75\ a_w$)	-	Molasses, soft dried figs, heavily salted fish, jam marmalade
0.70 - 0.60	-	-	Parmesan cheese, dried fruit, corn syrup, licorice
0.60 - 0.50	-	-	Chocolate, confectionery, honey, spices, noodles
0.40	-	-	Dried eggs, cocoa
0.30	-	-	Dried potato flakes, potato crisps, crackers, cake mixes, pecan halves
0.20	-	-	Dried milk, dried vegetables, chopped walnuts

The preservation of foods by moisture control may be enhanced by the use of other parameters that are inhibiting to the growth of microorganisms. Such "microbial hurdles" include the control of temperature and atmosphere in terms of processing and storage, pH, and the addition of chemical preservatives.

Temperature fluctuations and gradients must be minimized to prevent moisture migration within the product. If moisture migration occurs, the a_w throughout the food will no longer be uniform. The resultant areas of high and low a_w in the food system can change the growth rate of microorganisms.

Sorption Isotherms

A hydrated food can be dehydrated to remove moisture until the desired a_w is reached (desorption) or completely dehydrated and then rehydrated to the desired a_w (adsorption). A food is more stable against microbial spoilage if its a_w is adjusted by adsorption rather than by desorption.

Adsorption and desorption isotherms for a food system at a given temperature are plotted using a_w along the horizontal axis and % H_2O along the vertical. When plotted, the phenomenon of hysteresis can be observed. Hysteresis represents the difference in a_w between the adsorption and desorption isotherms (Figure 9-1).

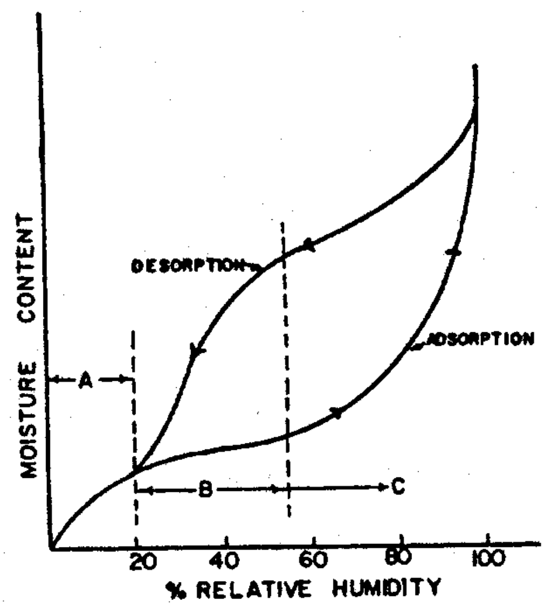

Figure 9-1

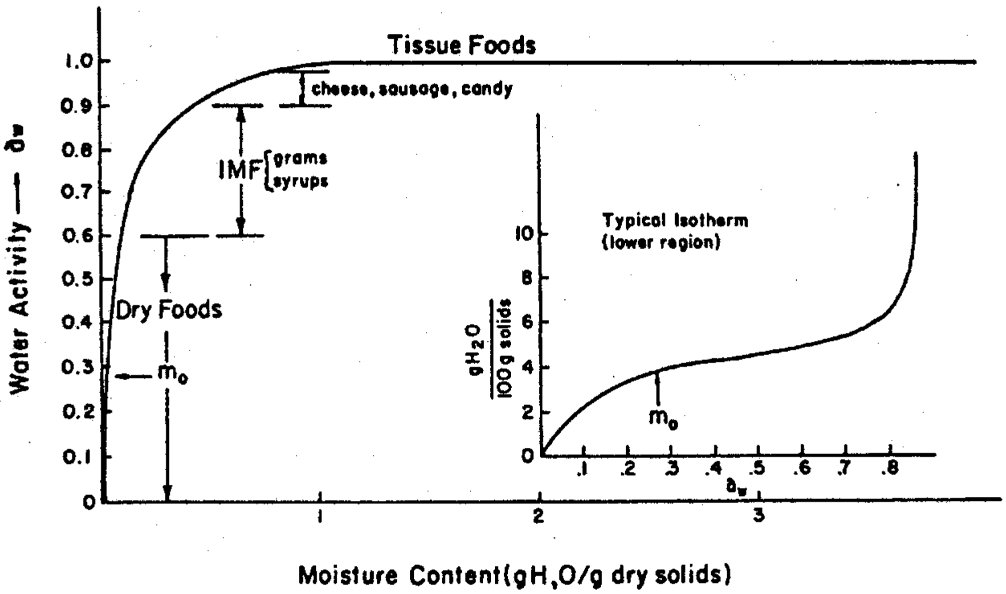

Figure 9-2: The range of moisture contents for some common foods and the low-moisture region for a typical isotherm

Effect of a_w on microbial growth

All microorganisms have an optimum and minimum a_w for growth. In general, most microorganisms can grow up to an $a_w = 0.99$. As the a_w is decreased, the rate of microbial growth will decrease until the minimum a_w is reached at which point growth ceases.

Most spoilage bacteria will not grow at an $a_w < 0.90$. Most yeasts cease growing at an $a_w < 0.85$ and most molds will not grow at an $a_w < 0.70$. Below an $a_w < 0.62$ no microbial growth is known to occur.

Microorganisms capable of growing at low a_w are able to accumulate intracellular compounds that are not harmful to the cell, but are capable of counteracting the osmotic imbalance between the cell and the high solute concentration of its environment. For example, at low a_w, halophilic bacteria accumulate K^+, yeasts accumulate polyols, and osmophilic bacteria accumulate proline.

Table 9- 3: Effect of a_w on Spoilage of Foods by Microorganisms

a_w	Probable Spoilage Organisms	Food
0.90-1.00	Bacteria	Cottage cheese, fresh meat
0.85-0.90	Bacteria and molds Yeasts	Margarine Sweetened condensed milk
	Bacteria	Whipped butter
0.80-0.85	Yeasts	Chocolate syrup, fruit syrup
0.75-0.80	Molds and yeasts	Jams
0.70-0.75	Yeasts	Confections
0.65-0.70	Osmophilic yeasts	Molasses
0.60-0.65	Xerophilic molds Osmophilic yeasts	Dried fruit Honey

Dehydrated or dried foods

Foods with an $a_w < 0.62$ are termed dehydrated. Dehydrated foods are stable against microbial spoilage, but not against enzymatic and non-enzymatic reactions which may occur and cause the dehydrated foods to deteriorate.

Intermediate Moisture Foods (IMF)

With the exception of yeast and mold growth, foods that are as stable as dried foods, in terms of microbial spoilage, but are too moist to be considered dry, are classified as IMFs. Like dehydrated foods, IMFs do not have to be refrigerated. IMFs have an a_w range of approximately 0.75 - 0.85 and contain 20 - 30% moisture. They contain a concentration of solutes that is sufficient to lower the a_w of the food system, yet is capable of maintaining a high moisture content for aesthetic appeal. Jams and semi-moist pet foods are examples of IMFs.

The use of many methods to control microbial growth can be demonstrated in the formulation and processing of a semi-moist pet food. The meat is first pasteurized to kill vegetative bacterial cells. The following ingredients are added for the reasons indicated:

Table 9- 4

Ingredient	Function
Propylene glycol	Plasticizer, humectant, antimycotic
K-sorbate	Antimycotic
Animal fat and emulsifiers	Plasticizer
Sugar, sorbitol, NaCl	Humectant
Soy flakes	Protein
Dry skim milk	Nutrients, humectant
Calcium	Nutrients

Antioxidants are added to pet foods and they are sometimes vacuum packed to further enhance the product's shelf life. The Food and Drug Administration (FDA) has recognized the importance of a_w in the preservation of foods by including the concept of a_w in its Good Manufacturing Practice Regulations (GMP). These regulations ensure against microbial spoilage, but not against enzymatic or chemical deterioration (see Appendix).

Procedure

An example of a totally dehydrated food and an IMF will be studied in five different relative humidity environments using five different saturated salt solutions. Since equilibrium relative humidity (ERH) and a_w are related, saturated salt solutions will be used to establish the ERH of the five "environments" we will study. Foods with two different initial water contents and initial water activities will be placed in these five environments and changes in their moisture content will be followed as they equilibrate to the water activity of their environment.

Reagents

K-acetate
$Mg(NO_3)_2$
NaCl
KCl
KNO_3

Part 1: Prepare ERH Chambers

Five relative humidity chambers for **each** material will be prepared with the following saturated salts that will establish the required %RH: (a_w)

Solute	a_w	Potato g-salt/g-H_2O	Dog Food g-salt/g-H_2O
K-acetate	0.225	20/3	20/0
$Mg(NO_3)_2$	0.520	20/3	20/0
NaCl	0.755	5/5	5/5
KCl	0.845	5/5	5/5
KNO_3	0.930	5/5	5/5

1. Prepare 10 (2 x 5) ERH chambers using plastic containers as follows:
    a. Pre-label the individual chambers with the salt type (you should have two of each of five salts), group number, and lab section.
    b. Put the required amount of salt and water into the corresponding plastic container.
    c. Into each plastic container, invert a metal weighing pan in which 3 triangles have been cut. Your weighing dish will rest on the inverted metal pan.
    d. Place the lid on the container. Over time, the corresponding ERH will be reached within the enclosed environment you have created.

Part 2: Dehydrated food sample (dried potato flakes)

The potato flakes have been dehydrated. Please tightly cover them when not in use so they are not exposed to the humidity in the room, as they will rapidly pick up moisture again in a humid atmosphere.

1. Weigh disposable weighing dish. **Record** weight of dish. **DO NOT TARE**. Calculate the "weight of the dish + 2.5 g". This gives you a guide of the weight to aim for to have 2.5 g of sample in the dish.
2. Using your target weight, weigh **approximately** 2.5 g potato flakes into dish. Record the **actual** initial weight of dish + sample.
3. Place sample in **appropriate** relative humidity chamber. Reseal the chamber. ***Be careful when you put the lid on the humidity chamber that you don't force a lot of air onto you flakes or they will fly everywhere!!!!!!!!!***
4. Repeat for other 4 samples. **Record which sample goes into which ERH chamber**.
5. You will return the following week to reweigh all your samples after storage. Record the final weight of dish + sample for each sample. **Make sure you have identified in which chamber each sample was stored.**

Part 3: Intermediate Moisture Food (dog food)

1. Weigh disposable weighing dish. **Record** weight of dish. **DO NOT TARE**. Calculate weight of dish + 4.0 g.
2. Weigh **approximately** 4.0 g of dog food into dish. Record the **actual** weight of dish + sample. Quickly mash the sample with a glass rod to increase surface area of the sample.
3. Place sample in appropriate relative humidity chamber. Reseal chamber.
4. Repeat for other 4 samples. **Record which sample goes into which ERH chamber**.
5. You will return the following week to reweigh your samples after storage. Record the final weight of dish + sample for each sample. **Make sure you have identified in which chamber each sample was stored.**
6. Note the initial moisture content of the dog food. This value will be provided for you by your TA.

NOTE: Throughout the course of the experiment the salt solutions in the relative humidity chambers must remain saturated in order to ensure that the a_w of each of the systems remains constant and known. If the food loses moisture to the atmosphere in the chambers such that the solution is no longer saturated, more salt must be added to the system to reestablish saturation. If the food gains moisture such that the solution dries up, water must be added to the system. The proportions of salt and water we specify have been calculated to prevent this, but be sure to check your chambers for saturation when you reweigh. The moisture content of the IMF has been allowed for in the instructions for setting up the humidity chambers.

Appendix: Definition of Terms and Calculation Hints

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

wt_i = initial sample weight (Sample weight - dish weight)

wt_f = final sample weight (Sample weight after adjustment to the selected water activity)
wt_d = dry sample weight (weight of sample at moisture content 0%)

X_{water} = fraction of water in sample

$X_{water} + X_{dry} = 1$ (the fraction of water, together with the fraction of dry solids, has to equal 1.)

wt_d = dry sample weight = $(1-X_{water})\, wt_i$
(the dry sample weight is the appropriate fraction of the **initial** wet sample weight)

For potato, initial sample weight equals dry sample weight. (ie $X_{water} = 0$)

% moisture final, dry weight basis $= \frac{wt_f - wt_d}{wt_d} \times 100$

% moisture change, dry weight basis $= \frac{wt_f - wt_i}{wt_d} \times 100$ **note that this can be + or –**

Appendix: Extract from FDA regulations

WATER ACTIVITY REQUIREMENTS of The Food and Drug Administration's good manufacturing practice regulations. Part 110 of the Code of Federal Regulations is quoted from FDA (1979c). Part 113 from FDA (1979a), and Part 114 from FDA (1979b).

21 CFR Part 110.80 (b) (15)

Foods such as, but not limited to, dry mixes, nuts, intermediate moisture foods, and dehydrated foods, that rely on the control of a_W for preventing the growth of microorganisms shall be processed to and maintained at a safe moisture level. Compliance with this requirement may be accomplished by any effective means including . . . :

(i) Monitoring the a_W of ingredients an finished products

(ii) Controlling the soluble solids-water ratio in finished products

(iii) Protecting finished foods from moisture pickup . . . so that the a_W . . . does not increase to an unsafe level.

21 CFR Part 110.3 (1)

"Safe-moisture level" is a level of moisture low enough to prevent growth of microorganisms in the finished product. The maximum safe moisture level for a food based on its water activity (a_W), will be considered safe for a food if adequate data are provided that demonstrate that the food at or below the given a_W will not support the growth of microorganisms.

21 CFR Part 113.3 (e) (1)

"Commercial sterility" . . . means the condition achieved ---

(i) By the control of water activity and the application of heat, which renders the food free of microorganisms capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution.

21 CFR Part 113.3 (n)

"Low-acid foods" means any foods . . . with a finished equilibrium pH greater than 4.6 and a water activity (a_W) greater than 0.85 . . .

21 CFR Part 113.81 (f)

. . . When normally low-acid foods require sufficient solute to permit safe processing at low temperatures, such as in boiling water, there shall be careful supervision to ensure that the equilibrium water activity (a_W) of the finished product meets that of the scheduled process. The scheduled thermal processes for foods having an a_W greater than 0.85 and less than the a_W that would allow the growth of spores of microorganisms of public health significance shall be sufficient to render the food free of microorganisms capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution.

21 CFR Part 113.100 (a)

. . .The following records shall be maintained:

(6) Food preservation methods wherein critical factors such as water activity are used in conjunction with thermal processing . . . and results of a_W determinations.

21 CFR 114.3 (b)

"Acidified foods" . . . have a water activity (a_W) greater than 0.85 and have a finished equilibrium pH of 4.6 or below . . .

21 CFR 114.3 (d)

"Low-acid foods" means any foods . . . with a finished equilibrium pH greater than 4.6 and a water activity (a_W) greater than 0.85 . . .