

# WATER ACTIVITY AND MICROBIAL STABILITY RELATIONSHIP IN A SEMI-PROCESSED HIGH PROTEIN FOODS

BY

UDENSI, E. O

TECHNOLOGY AND VOCATIONAL EDUCATION DEPARTMENT,  
ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY (ESUT)

## **Abstract**

*Post production storage affects both plant and animal products. Grains and meat products have available water under which spoiling micro-organisms cannot operate which is called water activity. Water activity is a factor in the water relations of micro-organism, food processing and storage. Its value is not only important in agriculture but in predicting the reactions of micro-organisms and the stability of food components. Many agricultural products are lost after harvest, be it crop, fish or meat. Investigating the approximate shelf-life of a semi processed high protein food product in the local markets at Enugu was necessitated by the experience of incessant power failure that had rendered many commercial cold rooms incapable of maintaining meat and fish products stable and without spoilage. The paper aimed at knowing the role played by water activity ( $a_w$ ) on its microbial stability and lowest limit of moisture content to which it would be dried for stability. Smoked beef weighing 150g was used as sample, employing vacuum desiccators for weight equilibration. Isopiestic equilibration  $a_w$  method was used. Results obtained showed that samples with  $a_w$  below 0.80 stored well and the total microbial counts was highest at sample with  $a_w$  0.97. Hence, the relationships between the loss of quality of foods and moisture content is centred on available water to micro-organisms in the semi-processed meat product.*

## **Introduction**

Water activity is described as the amount of unbound or free water available to support growth of micro-organisms, biological and chemical reactions in different foods. Therefore, this water requirement is referred to as available water or water activity ( $a_w$ ). It is expressed quantitatively, from the physical chemistry point of view, as vapour pressure of the solution (solutes in water in most foods) per the vapour pressure of the solvent, usually water. Thus, it is a ratio of vapour pressures of solution and solvent.

$$a_w = \frac{p}{p_0}$$

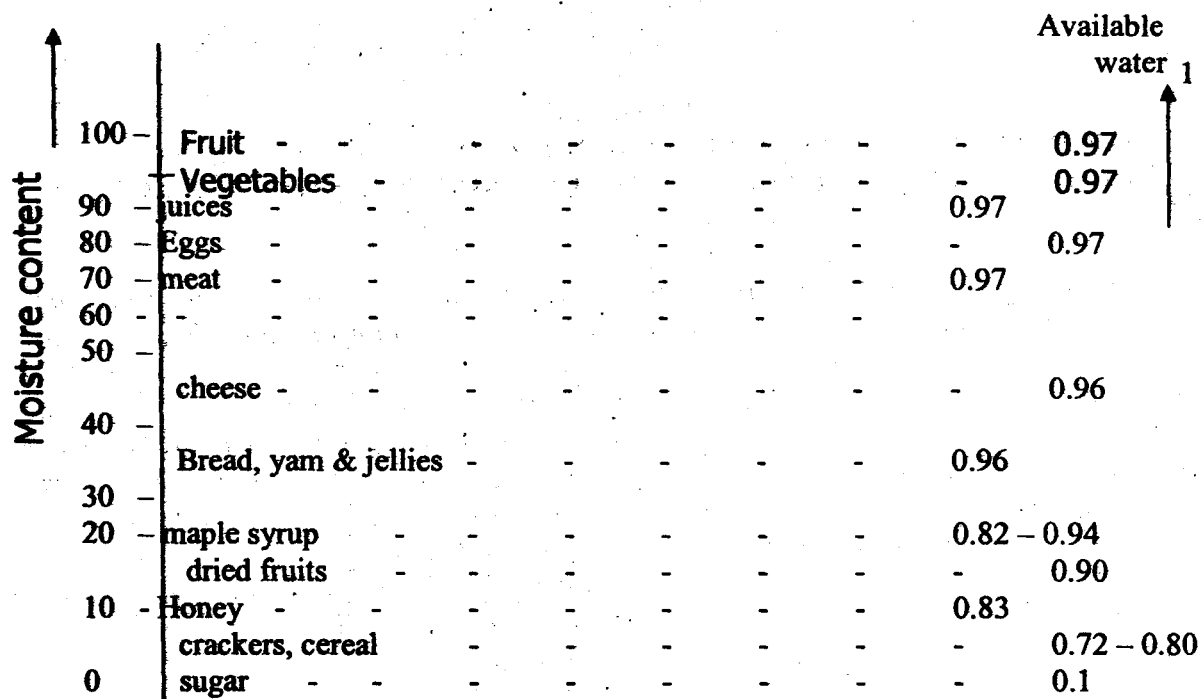
where  $a_w$  = water activity

$p$  = vapour pressure of solution

$p_0$  = vapour pressure of the solvent.

The growth of micro-organisms as a function of water activity has been of interest to agriculturists, microbiologists and food scientists. A desirable goal is the control of the available water of the foods, to a level which will prevent growth of food poisoning micro-organisms, yet allow enough moisture content for a mouth feel during the consumption of the food.

Water activity, not absolute water content, is what bacteria, enzymes and chemical reactants encounter and is affected by, at the micro-environmental level in food materials. Two foods with the same water content can have very different  $a_w$  values and vice versa, depending upon the degrees to which the water is free or otherwise bound to the food constituents.



**Fig 1: Water activity and Moisture content of some foods  
Kaplow (1970)**

It is known that extension of the shelf life of foods enhances ease of storage and convenient distribution. A major threat to food storage is the activities of micro-organisms. Traditionally, food spoilage could be delayed or prevented by implementing the preservation method of drying perishable foods, particularly flesh, like poultry, meat and fish. Traditionally preservation methods include smoking, sun-drying and salting. These methods, in principle, reduce the water activity of food products, thereby delaying the onset of microbial activity. These methods of food preservation are based on the fact that water is made unavailable for microbial growth; the relationship of  $a_w$  and microbial control has been known for decades now but putting this parameter to use as a tool was delayed due to the difficulty of measuring  $a_w$  or relative humidity of the food. Recently, many scientists have come out with different methods of  $a_w$  measurement and as a result many 'convenience' foods are now in the market, especially in the technologically advanced countries.

These traditional methods, highly practised by the masses in rural areas of Nigeria, are limited to sun drying, salting and smoking. They remove the bulk of the water as compared to freezing which converts the liquid to a solid ice state. The smoking presumably adds antimicrobial substances and plant phenolics that aid stabilizing effect of drying. The need arose to determine the water activity and microbial stability relationship in semi-processed high protein foods.

#### **Purpose of the Study**

The study specifically sought to:

1. Investigate the approximate shelf life of a semi processed high protein foods represented by smoked beef in the local markets at Enugu metropolis.
2. Find out the role played by water activity ( $a_w$ ) on foods microbial stability.

3. Find out the lowest limit of moisture content to which food product would be dried for stability.
4. Find the relationships between the loss of quality of foods and moisture content.

Beef is a common high protein food consumed in most parts of Nigeria and the state of this product has not received good attention under distribution to the final consumer as sold in the local markets. Water activity and microbial stability relationship in a semi processed (smoked) high protein food is a food preservation concept designed to find out the relative microbial stability of this beef product. The result could be used to help minimize the danger of spoilage due to inadequate processing and to reduce the health threatening activities of micro-organisms. Meat preservation and storage is to maintain them (the meat) in nutritionally wholesome and acceptable condition until they are consumed.

### Research Questions

The underlisted questions guided the researcher in the study:

- 1) What is the approximate shelf life of a semi processed high protein food product (smoked beef)?
- 2) What role does water activity ( $a_w$ ) play on smoked beef microbial stability?
- 3) What is the lowest limit of moisture content to which smoked beef would be dried for stability?
- 4) What are the relationships between the loss of quality of foods and moisture content?

### Research Methods

**Design of the study:** The design was experimental study

**Area of the study:** The study was done using smoked meat from Enugu North Local Markets (Aria Market) and Main Market.

**Sample:** The diced and smoke-dried beef was collected blended with blending machine, 150g was taken for the experiments.

### Materials and Methods

#### Equilibration of Samples

**Materials:** 8 vacuum desiccators, 8 evaporating dishes 150g of smoked meat, 8 75ml beakers and the underlisted chemicals whose  $A_w$  are indicated

$MgCl_2 \cdot 6H_2O$  -  $A_w$  0.33

$NaNO_2$  - 0.65

$NaCl$  - 0.75

$(NH_4)_2SO_4$  - 0.79

$K_2CrO_4$  - 0.88

$BaCl_2$  - 0.90

$KNO_3$  - 0.94

$K_2SO_4$  - 0.97

These  $a_w$  values were found at  $25^\circ C$  as indicated by Labuza et al (1996)

**Method:** With the concept that water activity is the equilibrium relative humidity or  $a_w$  at which substance neither gains nor loses moisture at a specific temperature 150g smoked beef was ground. 15g of the ground sample was weighed into each of the evaporating dishes and each dish placed in a vacuum desiccator. A saturated solution of each of the above chemical salts was prepared and taken with the 75mls capacity beakers which were each placed in the individual desiccators.

The desiccators were evacuated for 90 seconds and the contents allowed to equilibrate. After 24 hours the weight of the samples were determined. The desiccators were re-evacuated

and after a total of 72 hours, the weights were determined again. On the 3<sup>rd</sup> day of equilibration, the desiccators were loosened to let in air.

**Microbial Growth:** In confirming microbial stability of samples at various  $a_w$  values, the samples were cultured. 1g of each equilibrated sample was used for making the initial dilution. Four serial dilutions  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  were made. Pour plate method was used to obtain colony counts. Plating was done in duplicate and plates were incubated at 37°C and 25°C for bacterial and fungal counts respectively. The first microbial counts were obtained from plating of appropriate aliquots of 7-day equilibrated samples. The aerobic microbial counts were from plates incubated at 37°C and 25°C for 48 hours. Later on a two-week equilibrated samples was similarly treated and the corresponding results obtained.

**Moisture content:** 1 gram of a 9-day equilibrated sample was weighed into a moisture can. The samples were dried at 105°C for 24 hours to ensure enough drying.

The moisture determination was repeated after a total of 14 days of equilibration and dried at the same temperature for 24 hours.

## Results

### (a) The Equilibrated Samples

Equilibrium in this context is arbitrarily defined as a parameter representing the state of the food samples when held together in an enclosed chamber with salt solution of known water activity value for a period, so that such salt solution would influence that food sample by way of its water activity. The equilibrated samples yielded the following results:

**Table 1: Adsorption and desorption in a semi processed meat product at 24 and 72 hours equilibration**

$a_w$	Net difference after	Net difference after
	24 hrs	72 hours
0.33	- 0.9363	- 1.1150
0.65	- 0.1519	- 0.2668
0.75	- 0.0895	- 0.1342
0.79	+ 0.1480	+ 0.1707
0.88	+ 0.4110	+ 0.5273
0.90	+ 0.5260	+ 0.7227
0.94	+ 0.6547	+ 0.9417
0.97	+ 0.8698	+ 1.2370

(-) = net loss in weight (Desorption)

(+) = net gain in weight (Adsorption)

It could be seen that samples of  $a_w$  0.33, 0.65 and 0.75 were desorbing while samples of  $a_w$  0.79, 0.88, 0.90, 0.94 and 0.97 adsorbed. The adsorption and desorption gap increased with time (see values in above table).

From the concept that  $a_w$  at which equilibrium or constant weight is reached is the water activity of the product sample, this meat sample achieved its weight equilibrium at the  $a_w$  between 0.75 and 0.79. Therefore  $a_w$  of the control sample was probably in the range 0.75 to 0.79.

### 2. Storage Stability

Here, water activity of food shows that it could be used to predict the storage stability of such food. This 'stability' is the approximate length of time the smoked meat stored in the different levels of water activity at room temperature before microbial growth because evident in the samples including the control sample. The stability of the product is given below.

**Table 2: Storage Stability of Meat Samples Stored For 5 Weeks**

Samples $a_w$	Remarks
0.33	Stable for 5 weeks
0.65	Stable for 5 weeks
0.75	Stable for 5 weeks
0.79	At day 15, mouldy outgrowth and green colouration appeared.
0.88	At day 12 of incubation, microbial growth became evident in these two samples.
0.90	
0.94	Days, sample discoloured as a result of microbial growth.
0.97	The growth of micro-organisms was observed on the 7 <sup>th</sup> day.

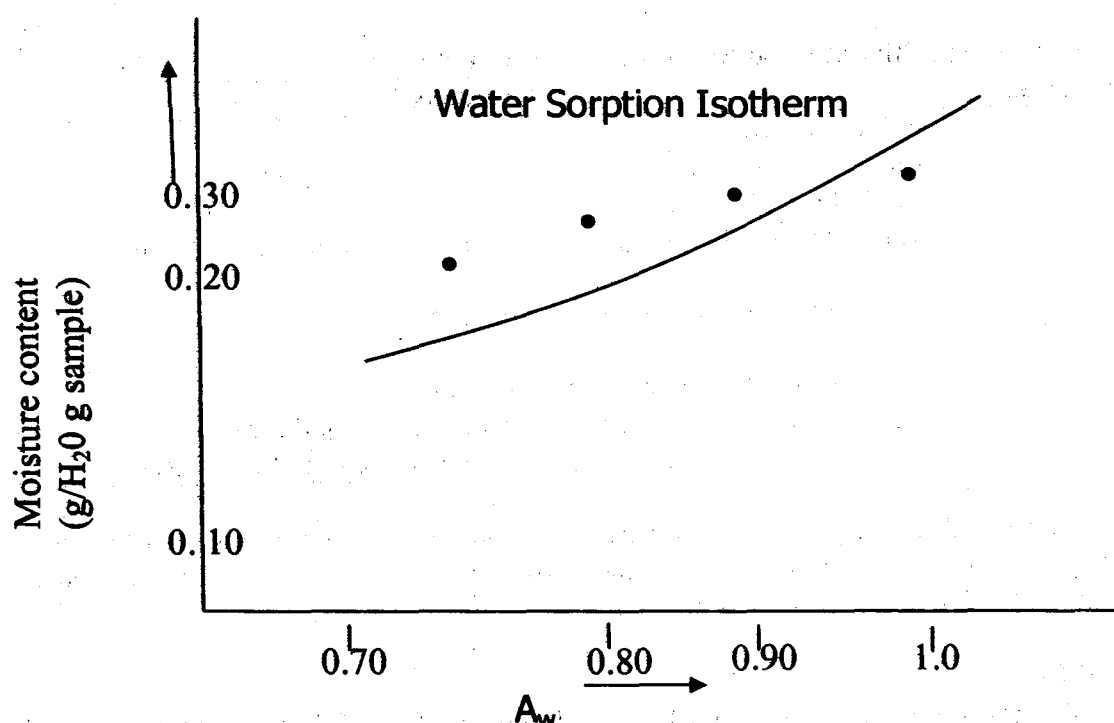
On the 15<sup>th</sup> day of equilibrium, powerful stale odours were coming out of the last four samples with  $a_w$  0.97; 0.94; 0.90; 0.88; in the above table. The control sample stored for 3 weeks and faint green colouration was noticed.

#### Microbial Counts

**Table 3: The total aerobic fungal and bacterial counts after 48 hours incubation at 25°C and of culturing at 37°C respectively.**

$A_w$ of the beef sample	Fungal counts of smoked beef	Bacterial counts of the smoked beef
0.33	< 30	< 30
0.65	< 30	$3.4 \times 10^2$
0.75	$3.2 \times 10^3$	$3.0 \times 10^5$
0.79	$3.2 \times 10^3$	$5.9 \times 10^5$
0.88	$3.6 \times 10^4$	$6.7 \times 10^5$
0.90	$4.3 \times 10^5$	$9.5 \times 10^5$
0.94	$6.5 \times 10^5$	$1.05 \times 10^6$
0.97	$1.04 \times 10^6$	$1.42 \times 10^6$

The microbial population increased with increasing water activity. The sample  $A_w$  0.97 recorded a fungal and bacterial counts of  $1.04 \times 10^6$  and  $1.42 \times 10^6$  respectively representing the maximum microbial growth while less than 30 recorded at 0.33 for both fungi and bacteria representing the microbial growth.



**Fig 2: Water content – water activity of smoked beef product after 2 weeks of Equilibration (Isopiestic) at different water activity levels.**

#### Moisture Content Result

With respect to food product at various percentage relative humidities or water activities, a different amount of water is bound. This relationship defines the moisture content in equilibrium with the different values of water activity at a particular temperature and is termed 'sorption isotherm' (fig 2).

Plotting the values of water activity against the corresponding moisture content gives a curve and the curves shows hysteresis, that is, the equilibrium moisture at a given  $a_w$  depends on (whether the food sample has  $a_w$  higher than the equilibrating solution or not) the direction the isotherm was made from. Therefore moisture content means the determination of the approximated water held in the food product.

**Table 4: Moisture content of smoked beef after 9 and 14 days of Equilibration and oven dried at 105°C for 24 hours.**

$A_w$	0.33	0.65	0.75	0.79	0.88	0.90	0.94	0.97
Day 9	0.0501	0.0801	0.1300	0.1307	0.1600	0.1700	0.2000	0.2099
net diff (g)								
Day 14 net	0.1200	0.1561	0.1900	0.1902	0.2100	0.2200	0.2500	0.3021
diff (g)								

Following readings obtained, the moisture content of each sample was dependent on its water activity value, in other words moisture content was higher at higher water activity and low at lower water activity ( $a_w$  0.33 and moisture content (M.C) 0.12g;  $a_w$  0.97 and M.C 0.30g).

At  $a_w$  greater than 0.75, the moisture content read almost the same value with 0.75. The case in point being  $a_w$  0.79, the reduction in water content was essentially derived by desorption – adsorption equilibrium during the equilibration.

## Discussion

The control of water content of a food product is a basic food processing technique that is based on the fact that the water content is decreased to a level to which microbial growth is prevented; therefore, the control of  $a_w$  levels in the food samples was done with the aid of suitable salts (humectants), which either increased or decreased the water activity values of the food samples enclosed in the dessicators. Thus, the adsorption and desorption of the smoked beef or food samples at different  $a_w$  levels were aids to determining the approximate water activity values of the samples.

Humectants are only suitable as an  $a_w$ -adjusting material in food if required concentration will guarantee safety and wholesomeness of such food (Troller and Christian, 1998). Therefore, salts used in equilibrating the meat sample at various  $a_w$  levels have been made to show that hydrated magnesium chloride, sodium nitrate and sodium chloride are better humectants as regards maintaining low water activity. Equilibrating the samples with the chosen salts revealed that meat samples with water activity of 0.75 or below were microbiological stable after five weeks of storage.

From the microbial plate counts, it was observed that the ability of micro-organisms to grow was reduced as the  $a_w$  was lowered and that  $a_w$  levels between 0.97 and 0.79 and temperature of 37°C, bacterial growth was optimum. Likewise the fungal counts were relatively high at the same  $a_w$  range for plates incubated at 25°C. Although I expected little or no growth below the  $a_w$  values of 0.25, the microbial spores in these samples might have germinated when the environment became conducive as a result of the moisture contributed by the culturing medium. It was observed that micro-organisms effected changes on the quality of the food products, and they can occur in two ways as a contributor to food preservation or to food spoilage and these effects depend on whether the changes are organoleptically desirable or undesirable. In beer manufacture, micro-organisms are used to produce very desirable products. In this study, products stored in environments with  $a_w$  greater than 0.80 gave organoleptically undesirable products in about two weeks.

## Principal Findings

The determination of water activity and moisture content led to the characterization of sorption behaviour of the meat product. It is proper to store a food product of low water activity in a manner as to enable it adsorb moisture, because adsorption increases the moisture content and water activity of such product and would encourage the onset of microbial activity.

Smoked meat (beef) product in the  $a_w$  range of 0.75 and 0.79, has about 20% moisture (table 4) and would be stable for a 3-week period as was observed in the control sample, after which microbial spoilage sets in and ends the production of organoleptically undesirable effects in the product. Hence smoked beef product that has about 20% moisture content would be stable for a 3-week period and should be consumed within the period to avoid health hazard.

## Conclusions

The importance of water activity-moisture content of foods cannot be over-emphasized, hence the important principles that explain the fundamental interactions of water within a food product in terms of storage stability are given as solute-water interactions and the capillary effect in a food. The relationship between loss of quality of food and its moisture content as best represented by water activity was demonstrated in this study. Smoked meat product at  $a_w$  0.75 or below stored better than those at higher water activities.

### Recommendations

- 1) Dried meat should be stored in an enclosure to prevent increasing  $a_w$  and onset of microbial spoilage.
- 2) Food product of low water activity should be consumed within a month of purchase for health reasons.

### References

- Frazier, W.C & Westhoff, D.C. (1998). *Food microbiology*. New York: McGraw – Hill.
- Harrigan, W.F. & McCance, M.E. (1996). *Laboratory methods in foods and dairy microbiology* (Revised Ed.). London: Academic Press Inc.
- Kaplow, M. (1970). Commercial development of intermediate moisture foods. *Food Technol.* (24) 889 – 893.
- Labuza, T.P., Acott, K; Tatini, S.R; Flink, J & McCall, W. (1996). Water activity determination: a collaborative study of different methods *J. Food Sci.* 41:910 – 917.
- Obanu, Z.A. (1996). Meat preservation at intermediate water activity. *Journal of food science and agriculture* 27:790 – 791.
- Troller, J.A. and Christian, J.H.B. (1998) *Water activity and foods*. New York: Academic Press Inc.,