

Protecting and improving the nation's health

Determination of water activity in food

National Infection Service Food Water and Environmental Microbiology Standard Method

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Status of National Infection Service Food, Water and Environmental Microbiology methods

These methods are well referenced and represent a good minimum standard for food, water and environmental microbiology. However, in using Standard Methods, laboratories should take account of local requirements and it may be necessary to undertake additional investigations.

The performance of a standard method depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

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Amendment history

Controlled document reference	FNES67 [P1]
Controlled document title	Determination of Water Activity in Food

The amendments that have occurred since the previous version of this document are shown below. On issue of revised or new documents each controlled document should be updated by the copyholder in the laboratory.

Page	Section(s) involved	Amendment	
All	All	Microbiology services replaced with National Infection	
		Service throughout	
10	References	Updated (CR11211)	

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Introduction

Scope

The method described is applicable to the determination of Water Activity (a_w) for all food types at a constant temperature.

Background

The water activity (a_w) of a food is a measure of availability of water for the metabolic activity and growth of microorganisms. Different species of microorganisms have different minimum levels of a_w that permit growth. The water activity of a food product can be used to predict microbial growth and to determine the microbial stability of a food product.

The measurement of a_w is usually performed using an electric hygrometer, which consists of a potentiometer, a sample/ sensor holder and a sensor. The a_w is a ratio so does not have units. Values range from 0.0 for a completely anhydrous sample to 1.0 for pure, salt-free water.

The water activity of most foodstuffs is from approximately 0.992 for untreated raw meat down to about 0.800 for salted and dried products. Generally, the growth of most bacteria and fungi occurs at a_w values above 0.90; if the a_w is below 0.8 the only organisms likely to grow are xerophilic moulds and osmophilic yeasts.

Although there can be a correlation between the total moisture content of food and its water activity, the correlation does not occur at all times. Food products can exist with high moisture content but have very little water activity. Ingredients can be added to a product to 'bind' the water making it unavailable for the growth of micro-organisms. A good example of a food product that contains quite a lot of water with a fairly low water activity is jam. Although composed of 50-60% water, jams usually have water activities around 0.75. The sugar and pectin in jams bind the water making it unavailable for microbial growth¹.

Meat products that utilize water activity as a way to increase shelf-stability include cured meats such as ham, fermented meats such as sausage and pepperoni and dried meats such as jerky. Most meat products with lower water activity levels utilize salt to bind water as well as drying techniques to lower the total moisture content of the product which in turn lowers the water activity. Appendix 1 shows water activity levels for bacterial growth / types of foods.

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This method is based on ISO 21807:2004², but has the following differences:

PHE method FNES67 (P1)	BS EN ISO 21807:2004 ²	Justification for variation
Standard deviation as per manufacturer's instructions for the instrument used.	Standard deviation of 0.002sn-1 for ranges 0.999 – 0.6000	Uncertainty of the measurements of 0.005 considered acceptable; all results are routinely reported to 2 decimal points.
Recalibration dependent on frequency of use	Recalibration daily using salt standards.	

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1. Principle

The required amount of sample is placed in a water activity sample vessel and is brought to ambient temperature. The sample is then placed within the air tight measuring cell of a calibrated water activity meter. Once the atmosphere within the cell has equilibrated, and the digital read-out has stabilised, the aw value can be recorded.

Precise control of temperature is very important when taking measurements. The equipment should be calibrated at the same temperature that is used for analysing the samples. The sample to be measured should be uniform in nature.

2. Definitions

Water activity (a_w)

For the purpose of this method, water activity is the vapour pressure of water in the aqueous phase of the test product divided by the vapour pressure of pure water or salt free water measured at a temperature of $25^{\circ}C\pm1^{\circ}C$. The ratio of water-vapour pressure in the food to the vapour pressure of pure water at the same temperature is expressed as a figure between 0.0 and 1.0.

3. Safety considerations

3.1 General safety considerations

Normal microbiology laboratory precautions apply³.

All laboratory activities associated with this SOP must be risk assessed to identify hazards⁴⁻⁵. Appropriate controls must be in place to reduce the risk to staff or other groups. Staff must be trained to perform the activities described and must be provided with any personal protective equipment (PPE) specified in this method. Review of this method must also include a review of the associated risk assessment to ensure that controls are still appropriate and effective. Risk assessments are site specific and are managed within safety organiser.

Information note: Throughout this method hazards are identified using **red text**. Where a means of controlling a hazard has been identified this is shown in **green text**.

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3.2 Specific safety considerations

Care must be taken when handling the humidity standards. COSHH assessments should be carried out for each chemical used, and appropriate protective clothing worn.

4. Equipment

Normal laboratory equipment required and in addition:

- water activity meter (various models)
- sample vessels
- calibration standards including at least 3 humidity values to cover the range of values commonly found in food (e.g. 11.3%, 32.8%, 52.9%, 75.3% and 90.1%)
- soft cloth
- cotton tip swab

5. Calibration and maintenance

5.1 Calibration

Calibration should follow the manufacturer's instructions for the equipment. The frequency of calibration required will depend on the frequency of use in each laboratory, as variation in humidity over time can fluctuate within the instrument and a reasonable period of calibration needs to be determined locally to ensure accuracy of results. In general, calibration on each day of use is considered good practice. At least 3 points must be calibrated using the salt standards, based on the expected range for the food types to be tested.

- Ensure humidity standards and measurement cell are at temperature equilibrium of 15°C 30°C without fluctuations.
- Allow the standard and measuring cell to equalize in temperature (approx. 30 minutes).
- Switch on the water activity meter and allow readings to be taken over 80 seconds (time elapsed is indicated on the digital display).
- If standard is reading the correct range for its relative humidity (Rh), there is no need to calibrate.
- If re-calibration is required (*i.e.* if Rh falls outside the specified range), perform this procedure following the manufacturer's instructions.

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• If new standards are purchased, Rh values must be deleted from the memory of the water activity meter so that the new results can be saved.

5.2 Cleaning of instrument

This should be carried out with a soft cloth and detergent (*e.g.*10% lipsol). If the measurement chamber is cleaned, ensure no liquid goes inside the instrument. Do not wet the connectors at the back of the machine. The instrument should be completely dry before it is put back into use.

5.3 Cleaning of infrared (IR) sensor

Clean the IR sensor with a slightly moist cotton tip swab. Do not press on the window of the IR-sensor. A contaminated IR-sensor leads to longer measurement times but not to wrong results.

6. Sample processing

6.1 Sample preparation and dilutions

Using sterile sampling equipment and the procedures described in standard method FNES26(F2)⁶ 'Preparation of samples and dilutions, plating and sub-culture', remove a homogenous portion of the food sample for water activity measurement into sample vessels. Fill the sample dishes as evenly as possible at least two thirds full and immediately replace the lid and ensure it is air tight.

Allow the temperature of the sample to come to ambient before testing. If the samples are not being tested immediately, store at approximate room temperature, ideally in an incubator to prevent temperature fluctuations which can alter water activity.

6.2 Sample examination

In order to test a sample, remove the lid of the sample dish and immediately place it in the measurement holder using tweezers and cover with the sensor to prevent water vapour affecting the true result of the product being tested. Allow the measuring cell to equilibrate (which can take 6-20 minutes or until the reading has stabilised), record the result. Further readings can be taken depending on the laboratory requirement and manufacturer's instructions. Once completed, remove the sample dish from the chamber and discard the product. Before testing further samples, the measuring cell should be left open to vent for a few minutes. Sample dishes can be removed, disinfected and dried before re-use.

7. Quality control

Daily testing of a_w standards is recommended to cover the range of a_w for sample typically tested. It is also recommended that laboratories performing the a_w test participate in an external proficiency scheme.

8. Calculation of results

Results are transferred to the StarLIms system as described in Method FNES6 (Q12)⁷. Sample processing and results entry in StarLims using the single result entry function. Results are recorded directly, and no calculations are required.

9. Reporting of results

All results are reported using the StarLims system as described in method FNES17 (Q13)⁸ Technical Validation and release of result in StarLims. The test report specifies the method used, all details necessary for complete identification of the sample and details of any incidents that may have influenced the result

Results should be recorded to 2 decimal places with no units as this is a ratio.

e.g. a_w 0.78

If necessary, quote the deviation factor as a result-based comment (± 0.005).

10. Acknowledgements and contacts

This Standard Method has been developed, reviewed and revised by National Infection Service, Food, Water and Environmental Microbiology Methods Working Group.

The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

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Appendix 1: Typical a_w values of food and the limits for microbial growth

Table 1 Typical aw values of some foods²

Water activity (a _w)	Food Type	
>0.98	Fresh meat & fish	
	Fresh Fruit & Vegetables	
	Milk & Cream	
	Fruit juices	
0.93 -0.98	Cooked sausages	
	Cheddar & Processed cheeses	
	Cured meat e.g. ham	
	Evaporated milk	
	Bread & crumpets	
0.85-0.93	Dry or fermented sausage	
	Dried beef	
	Raw ham	
	Mature cheddar cheese	
	Sweetened condensed milk	
0.60-0.85	Flour confectionary products e.g. cakes and	
	pastries	
	Dried fruit	
	Jams & jellies	
	Heavily salted fish	
	Extra mature cheese	
<0.60	Instant noodles	
	Sugar & chocolate confectionary products	
	Biscuits & crackers	
	Savoury snacks e.g. Crisps	
	Dehydrated vegetables	
	Cornflakes	

Table 2 aw limits for microbial growth

Organism	Optimum a _w
Campylobacter jejuni	>0.987
Vibrio cholerae	0.984
Vibrio parahaemolyticus	0.981
Staphylococcus aureus for growth (for enterotoxin production)	0.98
Clostridium perfringens	0.97
Escherichia coli	0.95
Salmonella spp.	0.94
Bacillus cereus	0.93
Listeria monocytogenes	0.92
Most other bacteria	0.91
Most yeasts	0.85
Aspergillus flavus for growth	0.82
Most other moulds	0.80
Halophilic Bacteria	0.75
Xerophilic Bacteria	0.65
Osmophilic yeasts	0.60

Appendix 2: Flowchart for performing a_w of food

Transfer a homogenous portion of the sample of food to a sample vessel and replace the lid

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Allow the sample temperature to equilibrate with room temperature.

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Switch on the aw meter and perform controls according to manufacturer's instruction

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Remove the lid from the sample vessel and place the sample in the humidity chamber

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Start measuring (6-20 mins). When result has stabilised record the result

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Open the chamber and remove the sample. Leave the chamber open for a few minutes before testing another sample