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Review
Section: Food Chemistry

Water Activity in Biological Systems – A Review

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Water deserves a major attention by researchers dealing with biological systems and related materials, like food, since it is ubiquitous and can be used like a "native" probe to garner information about the hosting system, provided it may be freely displaced across. Its thermodynamic potential, namely, the water activity, a_w, is related to that of the other compounds of the system considered *via* the Gibbs-Duhem relationship reflecting the extent of the residual availability of water to solvate further solutes and sustain the molecular mobility of the bio-polymeric compounds. As for the experimental approaches to a_w, this short review re-addresses the reader to other publications, while devotes a section to the Knudsen thermo-gravimetry that was used by the authors to determine the desorption isotherms of many food systems and related aqueous compounds. The paper remarks the importance of a preliminary assessment of water mobility and recalls the concept of "critical a_w" that takes into account the reduced mobility of water molecules in the vicinity of the glass transition. This opens the question of the reliability of sorption isotherms which encompass a wide a_w range and the interpretation of the observed adsorption/desorption hysteresis. The multi-phase character of many biological systems is another issue of interest related to the reliability of the experimental approaches to a_w. As examples of the role of a_w on the stability of bio-systems and on the practice of a technological treatment, protein unfolding and osmo-dehydration of fruit pulps are reported.

WATER ACTIVITY: A THERMODYNAMIC PROPERTY

Water activity, a_w, does play a pivotal role in the physiology of living organisms, may these be microbes, animals or vegetables, and the stability of products obtained from them. The main reason for such ubiquitous effect is the direct involvement of water in practically every process that can occur in biological systems, *e.g.*, transitions, chemical and biochemical reactions, diffusion, percolation, *etc.* Water indeed wets most of the surfaces and hydrates or solvates many chemical compounds, and, because of its large molecular mobility, can be displaced among the various compartments or phases that are normally present in biological systems, crossing interfaces, membranes and other layered supra-molecular structures under the effect of a single driving force: the gradient of its chemical potential, μ_w, which is related to a_w through the expression,

$$\mu_w = \mu_w^* + RT \ln a_w, \quad (1)$$

where " * " stands for pure compound and R and T are the gas constant and the absolute temperature, respectively. Even hydrophobic media or compounds have to comply with the presence of water, modifying their own structure or conformation, and affecting the structure of the surrounding water phase: a change that was dubbed *hydrophobic effect* [Privalov & Gill, 1989].

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Aqueous solutions allow an easy evaluation of a_w from the osmotic pressure, π, which can be determined experimentally in conditions of constant temperature, as π is directly related to a_w:

$$a_w = \exp(-\pi V_w / RT) \quad (2)$$

where V_w is the molar volume of pure water (18 10^{-6} m^3/mol at room temperature).

Unfortunately, such an approach is not feasible for most food and biological systems. One is therefore obliged to take advantage of the fact that a_w is related to the fugacity of water, f_w, which, in turn, may be approximated with the relevant partial pressure, p_w, (since this is sufficiently small):

$$a_w = f_w / f_w^* \sim p_w / p_w^* = RH\ 10^{-2} \quad (3)$$

where RH stands for relative humidity. Through a measure of p_w, one may therefore attain a_w, which, because of the expression (3), coincides with RH (a part form a factor 10^{-2}). For a review of the traditional methods used to determine water activity the reader is addressed to reference Barbosa-Cànovas *et al*. [2007], while a new approach, namely, the Knudsen thermo-gravimetry [Schiraldi & Fessas, 2003], is described in a dedicate section of this paper.

Thermodynamics of aqueous solutions

The Gibbs-Duhem expression allows evaluation of the thermodynamic activity of the solute, say glucose, a_G,

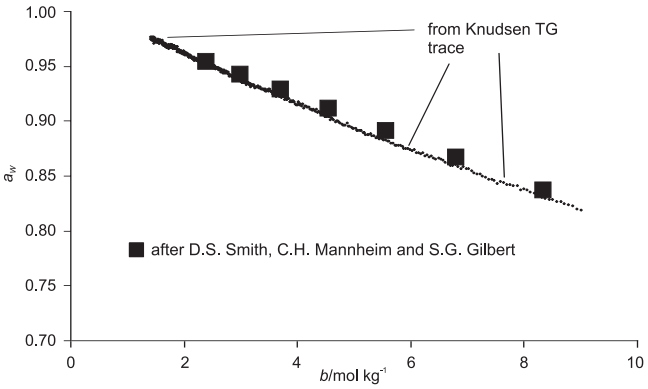

FIGURE 1. Water activity of aqueous glucose at various glucose molalities. The continuous line directly comes from a Knudsen TG run, while the dots are the data reported in reference [Smith *et al.*, 1981].

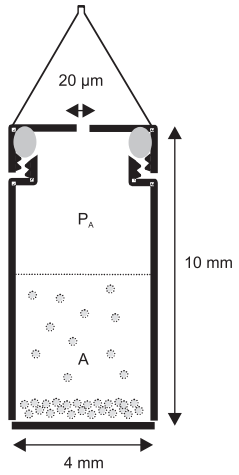

FIGURE 2. A schematic view of a Knudsen cell hanging in the thermobalance.

from the activity of the solvent, a_W, through integration of the expression,

$$\mathrm{d}\ln a_G = -\frac{X_W}{(1-X_W)}\mathrm{d}\ln a_W = -\frac{water\ mass}{(n_G \times M_W)}\mathrm{d}\ln a_W = \\ = -\frac{b_W}{b}\mathrm{d}\ln a_W \quad (4)$$

where X and b stand for molar fraction and molality, respectively, n_G is the number of moles of solute, M_W is the molar mass of water and $b_W = (10^3/18)$ mol/kg. The empirical fit of the a_W data collected at various b has to be obtained with a suitable (*e.g.*, polynomial) function, so that:

$$\mathrm{d}\ln a_W / b = [\mathrm{d}F(b) / \mathrm{d}b]\,\mathrm{d}b \quad (5)$$

A Knudsen isothermal desorption (see below) produces a very good basis (Figure 1) to find of a function like $F(b)$.

The evaluation of the thermodynamic activity of the solute requires definition of the integration limits. Since IUPAC recommends to choose the condition $b = 1$ with ideal behaviour as the standard state of a solute (see for example ref. [Pitzer, 1973]), the lower integration limit can be set at $b = 1$:

$$\ln\frac{a_G}{a_{G\,(b=1)}} = -b_W\int_1^b \frac{\mathrm{d}F(b)}{\mathrm{d}b}\mathrm{d}b \quad (6)$$

Taking into account the usual splitting of the thermodynamic activity, namely,

$$a_G = b \times \gamma_G, \quad (7)$$

this procedure allows evaluation of the activity coefficient, γ_G.

WATER ACTIVITY MEASUREMENT

Besides the osmo-meters, a number of instrumental approaches were so far proposed and used to determine *RH* [Barbosa-Cànovas, 2007]. Most of them require the achievement of some steady condition that implies a clear correspondence between moisture content, m_W / m_{dm} (m_{dm} being the dry matter mass) and *RH* of a given system. The series of collected *RH*-vs- m_{dm} isothermal data is used to define the sorption isotherm of the system investigated. Such methods require long equilibration times to achieve a single point: the whole sorption isotherm can require several days to be thoroughly assessed.

More recently, a much more convenient method was proposed [Schiraldi & Fessas, 2003] that allows simultaneous measure of both RH and moisture content: the Knudsen thermo-gravimetry (KTG) operating at constant temperature with Knudsen cells that replace the standard open pans of a thermobalance. The ideal Knudsen orifice has a diameter comparable to the mean free path of a gas molecule (1 – 10 µm, according to the pressure and temperature) and is pierced through an infinitely thin frame which has no walls: all the effusing molecules with a displacement component perpendicular to the orifice plane and trajectories that cross the orifice area can trespass the frame without changing their own speed [Wark *et al.*, 1967]. In a thermo-balance Knudsen cell such a frame actually is the cover of the cell that contains a volatile compound (Figure 2). The internal pressure of the cell, p_{in}, corresponds to the equilibrium vapour pressure of the volatile substance.

An isothermal Knudsen effusion implies a linear correlation between the mass flux and the pressure drop across the orifice,

$$F \propto (p_{in} - p_{out}) \quad (8)$$

If the volatile compound, A, is pure and $p_{out} >> p_{in}$,, then $p_{in} = p_A^*$ (where " * " stands for pure compound), at the temperature considered, and the effusion flux would correspond to a mass loss rate:

$$(\mathrm{d}m_A / \mathrm{d}t)^* \propto p_A^* \quad (9)$$

In the case of aqueous saturated salt solution, the partial pressure of water is constant at constant temperature, in the sense that it does not change if some solvent evaporates. As a consequence also the relevant water activity, $a_w = p_{sat} / p^*$, is constant (that is why these systems are chosen as reliable standards for the determination of a_w).

For saturated salt solutions too the mass loss during an isothermal Knudsen TG run would therefore occur at a constant rate,

$$(\mathrm{d}m_W / \mathrm{d}t)_{sat} \propto p_{sat} \quad (10)$$

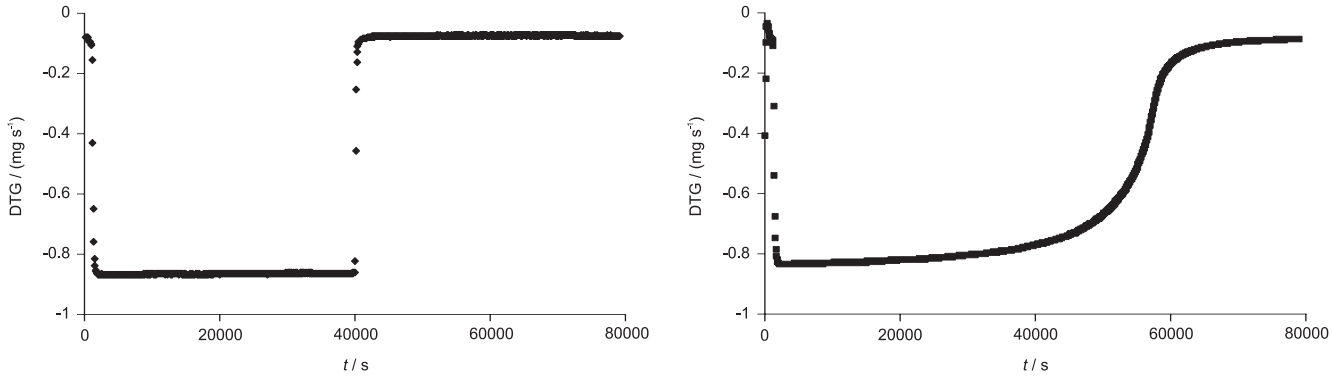

FIGURE 3. Knudsen isothermal desorption traces reported as DTG (time derivative of TG): (left) from pure water (a similar trend is observed for a saturated salt solution), (right) from a non saturated sample that modifies its composition.

In other words, the DTG trace (time derivative of the TG trend) of a KTG run is flat (Figure 3 left). Accordingly, one can determine the value of a_W of a given saturated salt solution as the ratio between the mass loss rates, one for the salt solution and the other for pure water, determined in two separate experimental runs at the same temperature and vacuum conditions, namely,

$$a_W = \frac{p_{sat}}{p^*} = \frac{(dm/dt)_{sat}}{(dm/dt)^*} \tag{11}$$

A constant mass loss rate however reflects a balance between the outbound flux of molecules through the cell orifice and the evaporation rate within cell. This steady condition does not necessarily mimic the true thermodynamic equilibrium, since it involves the molecular mobility through the liquid phase, the superficial tension of the liquid (with possible formation of bubbles) and, if the liquid phase is a saturated salt solution, perturbations related to the simultaneous segregation of the solute, not to say of stripping phenomena that can take place when the effusion flow is too high. It is therefore necessary to compare the observed behavior of salt saturated solutions with reference data, namely, the a_W values reported in the certified literature.

When water is desorbed from a not saturated system (Figure 3 right), the moisture content of the sample changes during the run and the relevant *DTG* trace shows a bending trend, as a_w decreases in the course of drying. At any time the residual moisture of the sample can be evaluated from the TG trace and expressed in any mass/mass concentration units (*e.g.*, water/dry-matter mass ratio, or molal concentration). One can therefore draw the desorption isotherm of a given system with a single Knudsen TGA run, that usually lasts 2–3 hours, with reference to a previous run performed with a sample of pure water in the same Knudsen cell, at the same temperature and vacuum regime.

In an isothermal Knudsen desorption performed at room temperature the moisture removed corresponds to the water fraction that has an easy access to the head space of the sample. A standard TG run (*i.e.*, with rise of the temperature say up to 200°C) performed at the end of the isothermal Knudsen desorption allows evaluation of the residual moisture that is unable to leave the sample at room temperature (*e.g.*, about 15% of the overall moisture of fresh bread crumb), in spite of the high dynamic vacuum conditions. This means that the Knudsen desorption does not involve water that is tightly bound to the substrate. In spite of this limitation, the Knudsen desorption isotherms allow some interesting observations. For example, if bread crumb samples are tested after different aging time, a drift toward lower RH is observed on aging, at any given content of removable moisture (Figure 4). This reflects the fact that even removable water experiences different binding forces, depending on the extent of crumb aging.

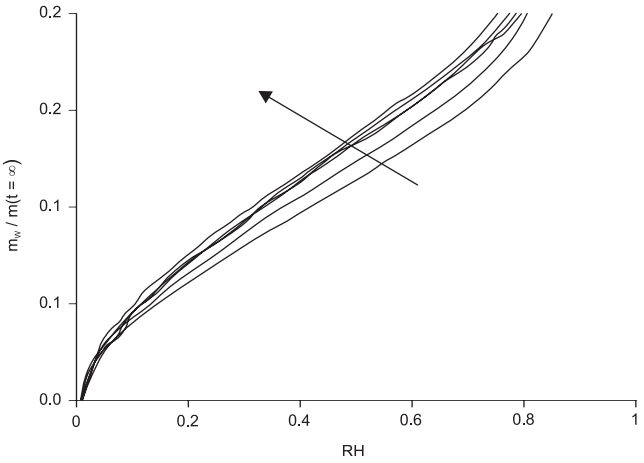

FIGURE 4. Desorption isotherms determined with Knudsen thermogravimetry for bread crumb samples of different age (from [Fessas & Schiraldi, 2005]).

Similar observations were reported for crumb of modified composition (*e.g.*, after addition of extra pentosans or globular proteins [Fessas & Schiraldi, 1998]).

WATER ACTIVITY AND WATER MOBILITY

As far as the experimental approach used deals (directly or indirectly) with the water partial pressure, the result should be referred to as *RH* rather than a_W, since the two quantities may actually coincide (a part form a factor 10^{-2}) only when the molecular mobility of water within the sample is adequate to sustain any displacement imposed by gradients of its chemical potential. This usually occurs for large a_W, *i.e.*,

$$\lim_{a_w \to 1} a_W = (RH)_{exp}\, 10^{-2}$$

In all the other cases,

$$(RH)_{exp}\, 10^{-2} \neq a_W$$

Appendix

On decreasing the water content of a real system, the corresponding water partial pressure, p_W, also decreases. Because of the drier conditions, a larger viscosity of the medium is expected, which can affect the evaluation of a_W, as the core-toward-surface migration of the moisture is reduced. For this reason the reliability of the evaluated a_W is poorer at the end of a desorption experiment.

It is therefore of some interest to single out a relationship between water migration rate, U_W, and "experimental" a_W. Thermodynamics of linear irreversible processes states that

$$U_W \approx u_W \cdot \Delta\mu_W = u_W \cdot RT \ln \frac{a_{W,i}}{a_{W,s}} \quad \text{(A1)}$$

where u_W and μ_W are the molecular mobility and the chemical potential of water, respectively, (the subscripts "i" and "s" stand for internal and superficial), R and T being the gas constant and the absolute temperature. If the water vapour is removed at a rate, dm / dt, one may imagine a steady state, where U_W counterbalances the removal of moisture,

$$U_W = dm / dt \quad \text{(A2)}$$

$$\text{and} \quad dm / dt = u_W [RT \ln (a_{W,i} / a_{W,s})] \quad \text{(A3)}$$

Equation (A3) allows one to recognize that

$$\lim_{u_W \to 0} (dm / dt) = 0 \quad \text{(A4)}$$

If the medium viscosity is very large, u_W is very small and, accordingly, dm / dt may become negligible for any $(a_{W,i} / a_{W,s})$ value. Conversely, a small drop of a_W between the core and the surface of the sample can produce a detectable migration rate that counterbalances the removal of water vapour when u_W is large. If the removal of water vapour is performed at a constant rate, as in the case of the effusion from a cell through a Knudsen orifice, then $dm / dt = K p_W$ (where K is a constant, see above).

When equation (A2) is matched with the equation (A1), one has:

$$u_W \cdot RT \ln \frac{a_{W,i}}{a_{W,s}} = \frac{p_W^{app}}{K} \quad \text{(A5)}$$

where p_W^{app} stands for the actual partial pressure of water within the cell and is related to an apparent water activity, namely,

$$a_W^{app} = \frac{p_W^{app}}{p_W^*} = \frac{K u_W}{p_W^*} \cdot RT \ln \frac{a_{W,i}}{a_{W,s}} \quad \text{(A6)}$$

Where the superscript " * " stands for "pure substance". At any given water content and temperature, this expression can be reasonably reduced to:

$$a_W^{app} \propto u_W \quad \text{(A7)}$$

Since the mobility of water is inversely proportional to the viscosity, η_W, experienced by water molecules that migrate from the core to the surface of the system, one may use equation (A7) to compare the apparent water activity, drawn from direct or indirect measures of p_W, to the "true" value of a_W, corresponding to the viscosity of pure liquid water, η_W^*

$$a_W = a_W^{app} (\eta_W / \eta_W^*) \quad \text{(A8)}$$

Expression (3) indeed implies attainment of a real equilibrium between sample bulk and relevant head space: a_W is a true bulk property of the sample, while $(RH)_{exp}$ is directly related to the adsorption/desorption at the sample surface. In other words, a_W can be reliably determined with a measure of p_W (and related physical properties of the head space, like dielectric constant, thermal conductivity, *etc*.), provided that water may actually be displaced throughout the system and attain the relevant head space, namely, the boundary with the surrounding atmosphere. If the molecular mobility of water is poor, a very long time is required to achieve such a condition and one may detect a lower apparent a_W. A simplistic treatment [see Appendix] allows prediction of a rough phenomenological correlation between apparent and actual water activity, namely:

$$a_W = a_{W,app} (\eta_W / \eta_W^*)$$

where η stands for viscosity ($\eta_W \geq \eta_W^*$).

Water activity and glass transition

What is more, when the temperature of the sample is below the relevant glass transition threshold, a real equilibrium is never reached (since below such a threshold the molecular mobility decreases by several orders of magnitude) and no reliable detection of a_W is possible, while an apparent *RH* may still be detected, because of the adsorption/desorption processes that take place at the surface and affect just few molecular layers beneath. Figure 5 reports a schematic view of the expected trend of the glass transition temperature, T_g, on varying the moisture content, or the corresponding *RH* at room temperature.

Accordingly, when one tries to approach a_W, which is a bulk property, through the measure of sorption processes, the latter must be actually governed by the underlying diffusion of water from the sample core toward the sample surface. If this is not the case, then the $(RH)_{exp}$ value may not correspond to the real a_W of the sample considered.

Since the glass transition threshold is raised up on dehydrating, any dehydration process unavoidably drives the samples across its glass transition, making the detection of a_W unreliable below the so-called [Roos, 1995; Maltini *et al*., 2003] critical a_W, namely the a_W value which is related to the moisture content that makes the glass transition temperature of the sample close to the room temperature (*i.e*., the usual operating conditions for the determination of a_W, see Figure 6).

This means that the widely diffused isothermal adsorption/desorption curves, determined for a number of products, actually reflect a bulk property for large (*e.g*., > 95%) *RH* and a surface property for small (*e.g*., < 50%) *RH*.

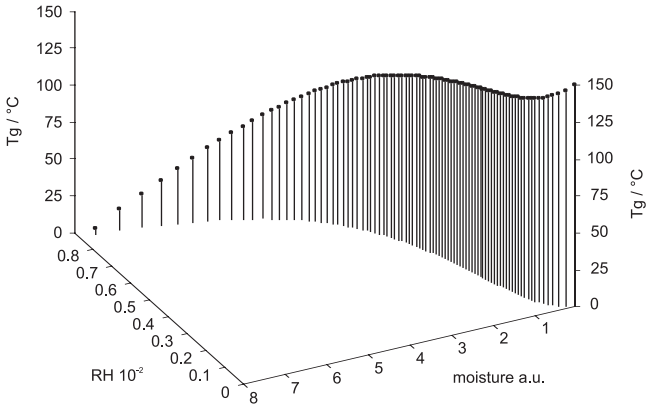

FIGURE 5. Schematic representation of the trend of RH (at room temperature) and that of the glass transition temperature *versus* the moisture content (in arbitrary units).

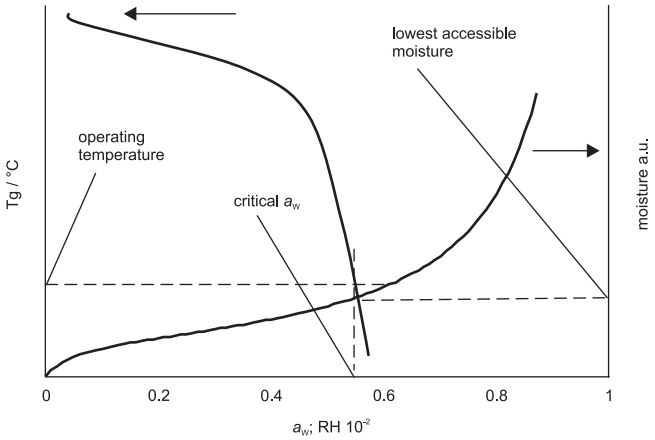

FIGURE 6. Identification of the critical water activity that defines the reliability limit of the isothermal sorption curve.

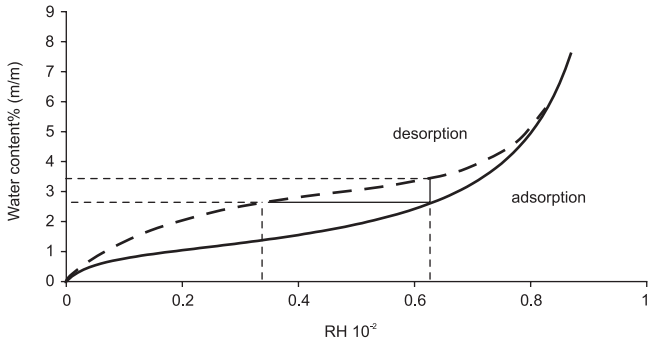

FIGURE 7. Hysteresis between adsorption and desorption isothermal trends.

The adsorption/desorption isotherms usually show an evident hysteresis (Figure 7). The gap between the adsorption and desorption curves reflects the fact that the path to achieve a given water content is different whether on adsorption or on desorption. It must be noticed that the state of the sample changes on scanning the *RH* range from 0 to 100% (or *vice versa*). In most cases, on adsorption for $RH < 0.25$, water is being actually fixed at the surface of the substrate and may migrate not further than 10–100 nanometers beneath this, even if the sample is left to "equilibrate" for months, as the temperature of the experiment is below the glass transition of the system. In these conditions, the apparent equilibrium that corresponds to the attainment of a steady sample mass does not imply a homogeneous partition of the moisture adsorbed, *i.e.*, $RH \neq a_W$. For $RH > 0.95$, the exchange of water does not involve the bare sample surface, since this is already well hydrated. Water is added onto previously fixed hydration layers that are closer to the surface of the substrate, or fill the bottom of its pores. As the sample is above its glass transition threshold, water can be displaced throughout the sample in few hours, *i.e.*, $RH \approx a_W$. This is evident in the case of homogeneous (stirred) aqueous solutions. For intermediate *RH* values, a longer time (typically, some days) is needed to equilibrate the system. These considerations hold also for the desorption trend, although in this case a more homogeneous distribution of the moisture is favoured.

On desorption the outer water layers are removed first, leaving back the more tightly bound ones. These are mainly trapped in small pores where, because of the surface tension, the partial pressure of water is lower than over a flat surface. The Kelvin equation predicts that in a pore of radius *r*, water activity is related to the wetting angle, θ, the surface tension, γ, and the molar volume, V_L, of the liquid:

$$a_W = \exp\left[-2\gamma V_L \cos\theta / rRT\right]$$

where *R* and *T* stand for the gas constant and absolute temperature, respectively. As a consequence, for a given water content, the observed *RH* is lower on desorption than on adsorption.

While the gap between the curves may be small (*e.g.*, 1%) as for the water content at a given *RH*, the difference can be much more substantial between the relevant *RH* values at a given water content, the partial pressure of water being lower in the head space of a sample undergoing desorption than in the head space of a sample undergoing adsorption.

A serious problem comes from the fact that many food products or biological tissues are phase separated or even divided in compartments that are not fully accessible. This means that within a given system one can find compartments with different *RH*, since natural barriers hinder the migration of water and prevent the attainment of a true thermodynamic equilibrium. As a possible consequence, an apparently well preserved food can conceal compartments where *RH* is large enough to sustain a detrimental microbial growth.

It can also happen that a wet surface may envelop a rather dry core, as the moisture is not allowed to diffuse in depth (because of a hydrophobic coating, or impermeable sets, *etc*.), or, *vice versa*, a rather dry outer layer may surround a more humid core (a bread loaf is an example). In such a case one may detect or try to detect the highest *RH* value, which will be relevant to the moistest compartment of the sample, and assume that the *RH* of all the others may not exceed it.

The above considerations suggest caution in using the available literature data on sorption isotherms, especially when they are collected in view of some technological application.

Issues of interest for a_W or *RH* in biological systems

It was so far understood that changes of either microbiological or biochemical and chemical nature must be expected in high *RH* conditions. Food is easily degraded when

RH is high, whereas it can be preserved at low *RH* (*e.g.*, dried, lyophilized, added with salt or sugars, *etc*.). This general statement is largely consolidated and put in practice in a number of industrial applications, but does not clarify the actual "mechanism" of such an effect. One has indeed to explain how a_W may affect the behavior of a given system. To summarise such wide subject, it is expedient to review the effects produced by changes of a_W on some important phenomena.

Water activity and stability of biological macromolecules

It is well known that protein unfolding is a substantially irreversible process, because of the aggregation of unfolded molecules. Nonetheless, in the vicinity of the transition temperature, one may describe the system with a thermodynamic model that assumes a two-states equilibrium. Some decade ago it was shown [Brandts, 1969; Privalov, 1990] that one may predict the occurrence of two different temperatures at which the Gibbs function of the native conformation, *N*, is equal to that of the unfolded conformation, *U* (or *D*, after some author). The higher one corresponds to the commonly experienced threshold of the *thermal denaturation*, T_d, while the lower one, T_L (usually below –10°C), is referred to as the temperature of *cold denaturation* (Figure 8).

Since the unfolding process is indeed governed by the displacement of the solvating water molecules, a more appropriate description of such a transition must include water [Schiraldi & Pezzati, 1992]:

$$N \cdot n\,H_2O \leftrightarrow U \cdot u\,H_2O + (n-u)\,H_2O \qquad (12)$$

At $T = T_U$, $\Delta_U G = 0$, where $\Delta_U G$ is the relevant drop of the Gibbs function. This means that the difference between the chemical potentials of the conformations *N* and *U* must be related to the water activity, namely,

$$\mu\,(U) - \mu\,(N) = (u-n)\,\mu\,(H_2O)$$

$$\Delta_U\mu = \delta \cdot \mu_W = (\delta \cdot \mu_W^*) + RT_U \ln (a_W)^\delta \qquad (13)$$

where $\delta = (u-n)$, namely the difference between the numbers of solvation water molecules for the conformation *U* and *N*, respectively. To highlight the effect of a_W, one may predict the value of T_U for different values of a_W, *e.g.*, close to or substantially lower than unity:

$$\ln (a_U / a_N) = -(\Delta_U G^\circ / RT) + \delta \cdot \ln a_W$$

$$\text{with } \Delta_U G^\circ = \Delta_U \mu^\circ - \delta \cdot u_W^* \qquad (14)$$

$$\text{and } \delta \cdot \ln a_W \geq 0, \text{ as } \delta < 0.$$

The unfolding equilibrium temperature, T_U, is usually referred to as the one at which $ln\ (a_U / a_N) = 0$. At this temperature,

$$(\Delta_U G^\circ / RT_U) = \delta \cdot \ln a_W \qquad (15)$$

For $ln\ a_W = 0$, the unfolding (either T_d or T_L) temperature is $\approx T_U'$ and $(\Delta_U G^\circ)_{T_U'} = 0$.

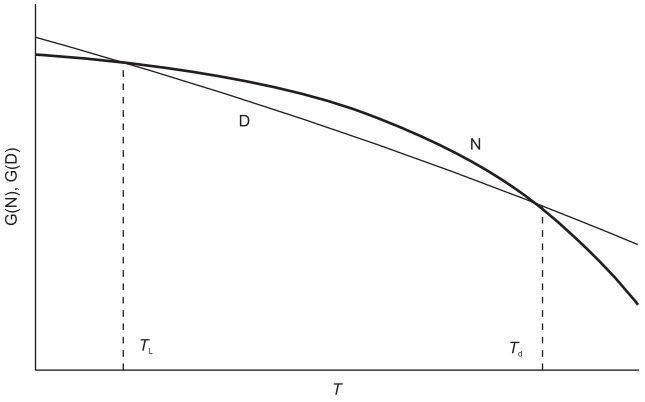

FIGURE 8. Gibbs function of native (N) and denatured (D) conformation of a protein in aqueous solution. The intersection points define the thermal and cold denaturation temperature, T_d and T_L.

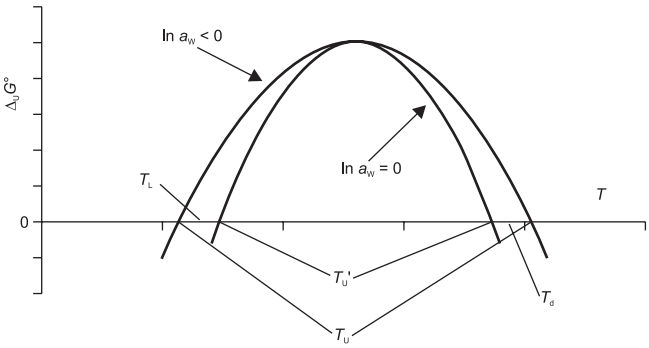

FIGURE 9. The unfolding temperature, T_U, of a protein in aqueous media changes with water activity, a_W. The two state model implies unfolding at two temperatures, T_d and T_L, that define the stability range of the native conformation. On decreasing a_W these limits move apart widening the stability $\Delta_U G$ bell.

When $ln\ a_W < 0$, the unfolding temperature is:

$$T_U = (\Delta_U G^\circ)_{T_U} / \delta \cdot R \ln a_W$$

This means that two different transition temperatures are expected, which are higher and lower than T_d and T_L, respectively, observed when $ln\ a_W \approx 0$. The difference between the transition temperatures is related to the corresponding entropy drop, which is positive at $T = T_d$, and negative at $T = T_L$:

$$(T_U - T_U') / T_U = \delta \cdot R \ln a_W / \Delta_U S^\circ \qquad (16)$$

In other words, the decrease of a_W implies a widening of the $\Delta_U G$-vs-T bell that encompasses the stability range of the native conformation of the protein (Figure 9).

A change of a_W can produce a substantial modification of the medium. To give an example, for a given concentration of a weak acid (base), the change of a_W affects the pH of the aqueous medium, since the actual expression for the dissociation constant does contain a_W (which is often approximated to the unity, as the condition of dilute solution is assumed):

$$k_a = [a\,(H_3O^+) \cdot a\,(A^-)] / [a\,(HA) \cdot a_W]$$

$$[k_a \cdot a\,(HA)] \approx a\,(H_3O^+)^2 / a_W$$

where HA is a monoprotic weak acid. One can easily obtain:

$$\mathrm{pH} \approx \alpha - \beta \ln a_{\mathrm{W}} \tag{17}$$

where $\alpha = 0.5\ [\mathrm{p}k_{\mathrm{a}} - \log_{10} a(\mathrm{HA})]$ and $\beta = 0.217$. When $a_{\mathrm{W}} \approx 1$, the above expression tends to coincide with the relationship reported in every school text of chemistry, where $a(\mathrm{HA})$ is replaced with the corresponding molar concentration. But if, for example, $a_{\mathrm{W}} = 0.75$ (which can be the case of many food products) the pH value would be 0.62 higher than the value calculated with the simplified expression $\mathrm{pH} = 0.5\ [\mathrm{p}k_{\mathrm{a}} - \log_{10} c(\mathrm{HA})]$. Similar effects can be easily predicted for weak bases and amphoteric compounds, like proteins. A change of a_{W} can therefore affect the medium pH, which can produce consequences on the dissociation degree of HA and, as in the case of proteins, on the molecular conformation, since it implies a change of the localized electric charges [van Holde *et al*., 1998].

The above description may also apply to other biological macromolecules, like carbohydrates and elongated proteins (like myosin, actin, gluten, collagen, *etc*.) and nucleic acids, which are indeed known to undergo substantial conformational changes because of aspecific interactions with the surrounding medium [Privalov & Khechinashvili, 1974].

These aspecific effects of a_{W} on the protein unfolding may be taken into account to explain how nature sustains the flexibility of life coping with a variety environmental conditions by means of buffer systems and osmotic solvent fluxes or active transfer of small mass solutes through cell membranes. An example, among many, is that of thermophilic bacteria which generally live in salt rich environments. It is indeed reasonable to expect the intracellular a_{W} of these organisms to be low and, as a consequence, the biochemical panoply of their enzymes to undergo unfolding and inactivation at higher temperatures than in mesophilic organisms.

Water activity and phase separation

Biological systems, including most food products, contain polymers that severely affect the overall physical properties even at concentrations as low as 0.5%. These substances, currently dubbed hydrocolloids, can trap large amounts of water with some (although not large [Fessas & Schiraldi, 2001]) effect on the value of a_{W}. In aqueous solutions the shape of the polymer molecules affects the solvating surface available for the interaction with water molecules which are linked to binding sites: however, there is an excess of "empty" binding sites with respect to the solvating water molecules. This excess mainly produces intra-molecular effects, like bridging bonds between binding sites that, because of the secondary or tertiary conformation of a given macromolecule, are close to each other, and inter-molecular aggregation. As a result, the properties of a given system (including food products) are mainly determined by the interactions between components, rather than by the peculiarity of single compounds [Tolstoguzov, 2003]. A typical event that takes place because of such interactions is phase separation.

Looking at biological system with the eyes of water, one understands that the solvent is engaged in a number of roles and, because of the large overall concentration of solutes, has a smaller chemical potential than pure water. A commonly used expression for μ_{W} is:

$$\mu_{\mathrm{W}} = \mu_{\mathrm{W}}^{*} + RT \ln a_{\mathrm{W}} \approx$$

$$\approx \mu_{\mathrm{W}}^{*} - RTV_{\mathrm{W}}^{*} \times (c / M + Bc^2 + \ldots) \tag{18}$$

where V_{W}^{*} is the molar volume of pure liquid water and B is the so-called second virial coefficient, c and M standing for the solute concentration and molecular mass, respectively. The right hand side of equation (18) is a view of the system through the eyes of the solute, since B reflects the interactions (solute-solvent and solute-solute) that produce the non-ideal behavior of the system. Equation (18) expresses the fact that the presence of solutes implies a decrease of a_{W}. This effect is however tuned by the second virial coefficient:

$$\ln a_{\mathrm{W}} = - V_{\mathrm{W}}^{*} [c / M + B \times c^2 + \ldots] \leq 0,$$

$$\text{or } B > - 1 / (c\,M) \tag{19}$$

which allows for either positive or negative values of B. If $B < 0$, the solvent power of water is poor, and for $B \approx 0$ the solute is separated as a precipitate (which can still fix some amount of water). Some solute however will remain in solution, although with a much smaller concentration. It is worth noting that B depends on pH and, in the case of proteins and charged amphoteric compounds, reaches a minimum (*i.e*., its maximum negative value) at the isoelectric point of the solute (where usually precipitation attains a maximum rate).

The formation of coexisting aqueous phases within a given system occurs because of the presence of thermodynamically incompatible water soluble macromolecules. In simple words, it may be said that different macromolecules compete with one another for the available water and tend to form aqueous phases of theirs own (Figure 10): each phase is largely enriched in a single macromolecule, while the concentration of the other polymers is vanishingly small [Tolstoguzov, 2003]. Solute-solute electrostatic and/or hindrance interactions can produce additional repulsion and attraction effects that can sustain the phase separation. Water activity has the same val-

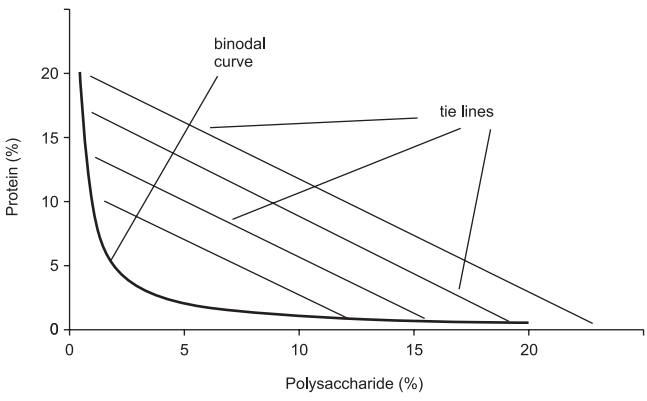

FIGURE 10. Phase diagram of two aqueous thermodynamically incompatible polymers. Above the binodal curve the system is split in two aqueous phase the composition of which is determined by the intercepts of the tie-lines with the binodal curve.

ue in every separated aqueous phase. To explain the process with the equation (18), it may be said that a polymer solute in a given aqueous phase has a strongly negative B because of its interactions with any dislike macromolecule; therefore only one polymer is allowed to remain within that phase. The excluded solutes do not necessarily precipitate: instead they form other aqueous phases where they can prevail. One example is the aqueous solution of a protein and a carbohydrate [Grinberg & Tolstoguzov, 1997], like gelatin and dextran, that splits in two aqueous phases which are rich in protein and in carbohydrate, respectively. One of these phases is finely (2–5 micrometer droplets) dispersed in the other that appears like a continuous matrix.

The same effect can be observed also between aqueous carbohydrate polymers, like amylose and amylopectin [Kalichevsky & Ring, 1987].

Fundamental studies on this subject considered polymer solutions with low (10^{-3} M) and very low (less than 10^{-4} M) solute concentrations. Since B of biopolymers usually is rather small, large changes of a_W produce minor effects on B. That is why most of these studies directly concern the solute properties and the solute-solute interactions (mainly through spectroscopic and NMR investigations). This is not the case of real biological systems and food products, where c can be rather large. Small changes of the moisture content can imply a large drop a_W (remind the shape of sorption isotherms for low water/dry matter mass ratios) and therefore produce large effect on the overall structure and organization of the system, especially when some shearing stress is applied [Tolstoguzov, 2003].

DEHYDRATION OF FOOD SYSTEMS

Dehydration is the most applied method to preserve food. Reducing the water content usually implies decrease of RH and therefore hindering of microbial spoilage and chemical or biochemical degradation. The extent of moisture release is usually related to the highest temperature experienced by the system. Mild treatments allow preservation of the most labile and nutritionally important compounds, but imply rather large residual moisture levels. It is therefore of interest to assess the value of RH attained at the end of the treatment.

A large removal of the moisture is achieved in dehydration of fruit pulps and syrups. These systems deserve investigation in view of some treatment used to prepare partially dehydrated products. An example is the so-called osmo-dehydration (Figure 11).

To characterise the system undergoing the treatment one does not need to define the whole desorption trend. It is instead necessary to assess the correlation between a_W and moisture content in a limited range, namely, between the a_W of the starting fruit pulp and the a_W of the sugar syrup used in the osmo-dehydration treatment. In these samples the water mobility is large enough to attain the thermodynamic equilibrium of water partition between intra- and extra-cellular environment. This allows the assumption that $a_W = RH\ 10^{-2}$. The Knudsen thermogravimetry can be used to mimic in a continuous way the dehydration process [Pani & Schiraldi, 2010]. If no damage of the cell membranes has

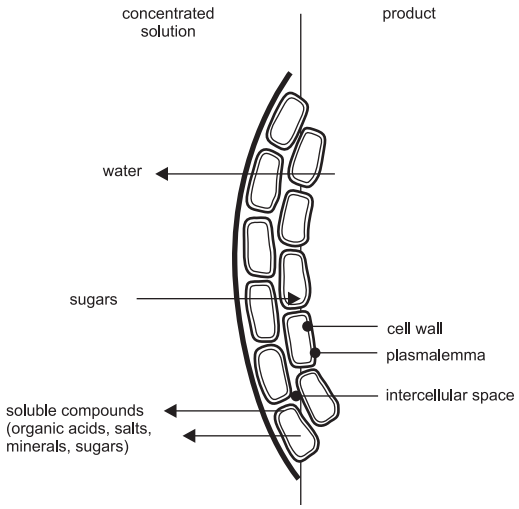

FIGURE 11. Matter flows involved in a osmo-dehydration treatment of fruit pulp.

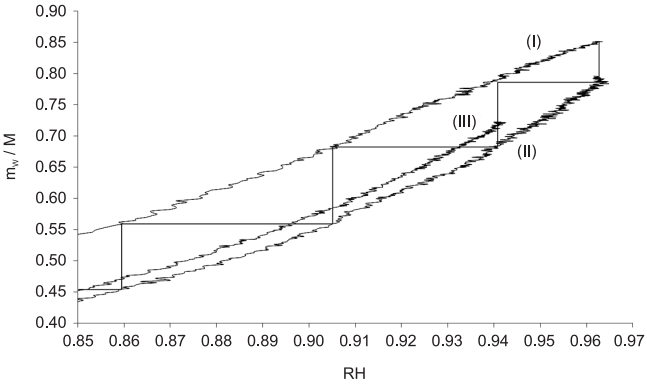

FIGURE 12. Knudsen desorption trend of apple pulp (I), hypertonic glucose syrup (II) and partially osmo-dehydrated apple pulp (III).

occurred, the water migration from the fruit cells toward the surrounding hypertonic sugar syrup requires 2–4 hours. One needs to define three desorption trends relevant to: (I) the non-treated fruit pulp, (II) the sugar syrup used in the process and (III) a partially osmo-dehydrated fruit pulp (Figure 12).

The trend (I) reflects the release of water from the fruit cells and therefore represents the state of water in the cytoplasmic environment. The trend (III) lays between the other two, since the water released from the fruit cells into the extra-cellular environment dilutes the syrup coming from the bath where the fruit has been poured for the treatment. This means that trend (III) reflects the state of water in the extra-cellular regions. At any dehydration level, water activity must be the same in the three phases. Looking at the dehydration trends (Figure 12), the gap between trends (I) and (II) and the position of the trend (III) in the middle can be used to predict the mass proportion between intra- and extra-cellular phases in corresponding partially osmo-dehydrated sample [Pani & Schiraldi, 2010]. On the other hand, if one considers a given overall moisture/dry matter mass ratio, the water activity of a heat dried sample, which too is represented by the trend (I), is always lower than that of an osmo-dehydrated fruit pulp.

CONCLUDING REMARKS

Water activity, a_w, is a thermodynamic potential energy that concerns any substance that may interact with water. Because of the ubiquity of this compound in biological organisms and related materials, like food, a_w plays a pivotal role in the overall behaviour of such complex systems. This provides an excellent opportunity for the investigators who may garner reasonable interpretations by choosing to observe the world with the eyes of water. Water is indeed a "native" probe compound that sends clear messages whenever it may freely move. This condition is indeed the only limit to the use of water properties to draw information about the hosting system. A preliminary step of any investigation dealing with water must therefore be the assessment of its actual molecular mobility. A too high viscosity of the medium can hinder displacements of water driven by gradients of its chemical potential and lead to erroneous evaluations of a_w and other correlated properties (see appendix).

As a warm recommendation for the reader, I suggest to perfect the knowledge of water properties starting with a molecular theory of water and aqueous solutions [Ben-Naim, 2009], as this can be of great help to understand biological systems.

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