

6 Review

7 Section: Food Chemistry

9 **Water Activity in Biological Systems – A Review**

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

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38 **WATER ACTIVITY: A THERMODYNAMIC PROPERTY**

40 Water activity, a_w , does play a pivotal role in the physiology of living organisms, may these
41 be microbes, animals or vegetables, and the stability of products obtained from them. The main
42 reason for such ubiquitous effect is the direct involvement of water in practically every process that
43 can occur in biological systems, *e.g.*, transitions, chemical and biochemical reactions, diffusion,
44 percolation, *etc.* Water indeed wets most of the surfaces and hydrates or solvates many chemical
45 compounds, and, because of its large molecular mobility, can be displaced among the various

46 compartments or phases that are normally present in biological systems, crossing interfaces,
 47 membranes and other layered supra-molecular structures under the effect of a single driving force:
 48 the gradient of its chemical potential, μ_w , which is related to a_w through the expression,

$$49 \mu_w = \mu_w^* + RT \ln a_w, \quad (1)$$

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

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

$$56 a_w = \exp(-\pi V_w / RT) \quad (2)$$

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

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

$$61 a_w = f_w / f_w^* \sim p_w / p_w^* = RH \cdot 10^{-2} \quad (3)$$

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

73 **Thermodynamics of aqueous solutions**

74 The Gibbs-Duhem expression allows evaluation of the thermodynamic activity of the solute,
 75 say glucose, a_G , from the activity of the solvent, a_w , through integration of the expression,

$$76 d \ln a_G = - \frac{X_w}{(1-X_w)} d \ln a_w = - \frac{\text{water mass}}{n_G \times M_w} d \ln a_w = - \frac{b_w}{b} d \ln a_w \quad (4)$$

78 where X and b stand for molar fraction and molality, respectively, n_G is the number of moles of
79 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
80 collected at various b has to be obtained with a suitable (*e.g.*, polynomial) function, so that:

81
82
$$d \ln a_W / b = [dF(b)/db] db \quad (5)$$

83
84 A Knudsen isothermal desorption (see below) produces a very good basis (Figure 1) to find of a
85 function like $F(b)$.

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

90
$$\ln \frac{a_G}{a_{G(b=1)}} = -b_W \int_1^b \frac{dF(b)}{db} db \quad (6)$$

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

92
93
$$a_G = b \times \gamma_G, \quad (7)$$

94
95 this procedure allows evaluation of the activity coefficient, γ_G .

96

97 WATER ACTIVITY MEASUREMENT

98

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

106 More recently, a much more convenient method was proposed [Schiraldi & Fessas, 2003]
107 that allows simultaneous measure of both RH and moisture content: the Knudsen thermo-
108 gravimetry (KTG) operating at constant temperature with Knudsen cells that replace the standard
109 open pans of a thermo-balance. The ideal Knudsen orifice has a diameter comparable to the mean
110 free path of a gas molecule (1 – 10 μm , according to the pressure and temperature) and is pierced

111 through an infinitely thin frame which has no walls: all the effusing molecules with a displacement
 112 component perpendicular to the orifice plane and trajectories that cross the orifice area can trespass
 113 the frame without changing their own speed [Wark *et al.*, 1967]. In a thermo-balance Knudsen cell
 114 such a frame actually is the cover of the cell that contains a volatile compound (Figure 2). The
 115 internal pressure of the cell, p_{in} , corresponds to the equilibrium vapour pressure of the volatile
 116 substance.

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

$$119 \quad F \propto (p_{in} - p_{out}) \quad (8)$$

120
 121 If the volatile compound, A, is pure and $p_{out} \ll p_{in}$, then $p_{in} = p_A^*$ (where “ * ” stands for pure
 122 compound), at the temperature considered, and the effusion flux would correspond to a mass loss
 123 rate:
 124

$$125 \quad (dm_A/dt)^* \propto p_A^* \quad (9)$$

126
 127 In the case of aqueous saturated salt solution, the partial pressure of water is constant at constant
 128 temperature, in the sense that it does not change if some solvent evaporates. As a consequence also
 129 the relevant water activity, $a_w = p_{sat}/p^*$, is constant (that is why these systems are chosen as reliable
 130 standards for the determination of a_w).
 131

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

$$134 \quad (dm_W/dt)_{sat} \propto p_{sat} \quad (10)$$

135
 136 In other words, the DTG trace (time derivative of the TG trend) of a KTG run is flat (Figure 3 left).
 137 Accordingly, one can determine the value of a_w of a given saturated salt solution as the ratio
 138 between the mass loss rates, one for the salt solution and the other for pure water, determined in two
 139 separate experimental runs at the same temperature and vacuum conditions, namely,
 140

$$141 \quad a_w = \frac{p_{sat}}{p^*} = \frac{(dm/dt)_{sat}}{(dm/dt)^*} \quad (11)$$

142
 143

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

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

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

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

173

174 **WATER ACTIVITY AND WATER MOBILITY**

175

176 As far as the experimental approach used deals (directly or indirectly) with the water partial
177 pressure, the result should be referred to as *RH* rather than a_w , since the two quantities may actually

178 coincide (a part from a factor 10^{-2}) only when the molecular mobility of water within the sample is
179 adequate to sustain any displacement imposed by gradients of its chemical potential. This usually
180 occurs for large a_w , *i.e.*,

$$\lim_{a_w \rightarrow 1} a_w = (RH)_{\text{exp}} 10^{-2}$$

184 In all the other cases,

$$(RH)_{\text{exp}} 10^{-2} \neq a_w$$

186
187 Expression (3) indeed implies attainment of a real equilibrium between sample bulk and relevant
188 head space: a_w is a true bulk property of the sample, while $(RH)_{\text{exp}}$ is directly related to the
189 adsorption/desorption at the sample surface. In other words, a_w can be reliably determined with a
190 measure of p_w (and related physical properties of the head space, like dielectric constant, thermal
191 conductivity, *etc.*), provided that water may actually be displaced throughout the system and attain
192 the relevant head space, namely, the boundary with the surrounding atmosphere. If the molecular
193 mobility of water is poor, a very long time is required to achieve such a condition and one may
194 detect a lower apparent a_w . A simplistic treatment [see Appendix] allows prediction of a rough
195 phenomenological correlation between apparent and actual water activity, namely:

$$a_w = a_{w,\text{app}} (\eta_w / \eta_w^*)$$

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

201 **Water activity and glass transition**

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

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

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

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

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

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

$$a_w = \exp[-2\gamma V_L \cos \theta / rRT]$$

244
245 where R and T stand for the gas constant and absolute temperature, respectively. As a consequence,
246 for a given water content, the observed RH is lower on desorption than on adsorption.

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

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

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

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

264

265 **Issues of interest for a_w or *RH* in biological systems**

266 It was so far understood that changes of either microbiological or biochemical and chemical
267 nature must be expected in high *RH* conditions. Food is easily degraded when *RH* is high, whereas
268 it can be preserved at low *RH* (*e.g.*, dried, lyophilized, added with salt or sugars, *etc.*). This general
269 statement is largely consolidated and put in practice in a number of industrial applications, but does
270 not clarify the actual “mechanism” of such an effect. One has indeed to explain how a_w may affect
271 the behavior of a given system. To summarize such wide subject, it is expedient to review the
272 effects produced by changes of a_w on some important phenomena.

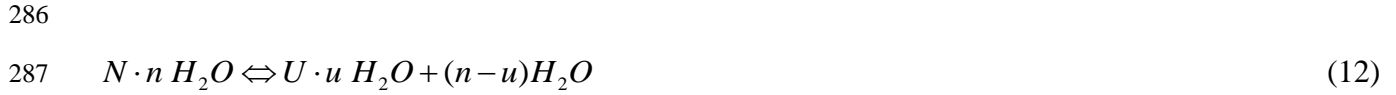
273

274 **Water activity and stability of biological macromolecules**

275 It is well known that protein unfolding is a substantially irreversible process, because of the
276 aggregation of unfolded molecules. Nonetheless, in the vicinity of the transition temperature, one
277 may describe the system with a thermodynamic model that assumes a two-states equilibrium. Some
278 decade ago it was shown [Brandts, 1969; Privalov, 1990] that one may predict the occurrence of
279 two different temperatures at which the Gibbs function of the native conformation, *N*, is equal to
280 that of the unfolded conformation, *U* (or *D*, after some author). The higher one corresponds to the

281 commonly experienced threshold of the *thermal denaturation*, T_d , while the lower one, T_L (usually
 282 below -10°C), is referred to as the temperature of *cold denaturation* (Figure 8).

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



287
 288
 289 At $T = T_U$, $\Delta_U G = 0$, where $\Delta_U G$ is the relevant drop of the Gibbs function. This means that the
 290 difference between the chemical potentials of the conformations N and U must be related to the
 291 water activity, namely,

$$292 \quad \mu(U) - \mu(N) = (u - n)\mu(H_2O)$$

$$293 \quad \Delta_U \mu = \delta \cdot \mu_w = (\delta \cdot \mu_w^*) + RT_U \ln(a_w)^\delta \quad (13)$$

294
 295 where $\delta = (u - n)$, namely the difference between the numbers of solvation water molecules for the
 296 conformation U and N , respectively. To highlight the effect of a_w , one may predict the value of T_U
 297 for different values of a_w , *e.g.*, close to or substantially lower than unity:

$$298 \quad \ln(a_U / a_N) = -(\Delta_U G^\circ / RT) + \delta \cdot \ln a_w$$

with

$$299 \quad \Delta_U G^\circ = \Delta_U \mu^\circ - \delta \cdot \mu_w^* \quad (14)$$

and

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

300
 301 The unfolding equilibrium temperature, T_U , is usually referred to as the one at which $\ln(a_U/a_N) = 0$.

302 At this temperature,

$$303 \quad (\Delta_U G^\circ / RT_U) = \delta \cdot \ln a_w. \quad (15)$$

304
 305
 306 For $\ln a_w = 0$, the unfolding (either T_d or T_L) temperature is $\square T_U'$ and $(\Delta_U G^\circ)_{T_U'} = 0$

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

308

309 $T_U = (\Delta_U G^\circ)_{T_U} / \delta \cdot R \ln a_w.$

310

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

315

316 $(T_U - T_U') / T_U = \delta \cdot R \ln a_w / \Delta_U S^\circ$ (16)

317

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

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

324

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

325

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

326

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

328

329 $\text{pH} \approx \alpha - \beta \ln a_w$ (17)

330

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

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

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

351

352 **Water activity and phase separation**

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

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

368

$$369 \mu_w = \mu_w^* + RT \ln a_w \approx \mu_w^* - RTV_w^* \times (c/M + Bc^2 + \dots) \quad (18)$$

370

371 where V_w^* is the molar volume of pure liquid water and B is the so-called second virial coefficient,
372 c and M standing for the solute concentration and molecular mass, respectively. The right hand side
373 of equation (11) is a view of the system through the eyes of the solute, since B reflects the

374 interactions (solute-solvent and solute-solute) that produce the non-ideal behavior of the system.
375 Equation (11) expresses the fact that the presence of solutes implies a decrease of a_w . This effect is
376 however tuned by the second virial coefficient:

$$378 \ln a_w = -V_w^* [c/M + B \times c^2 + \dots] \leq 0, \text{ or } B > -1/(c M) \quad (19)$$

379
380 which allows for either positive or negative values of B . If $B < 0$, the solvent power of water is poor,
381 and for $B \ll 0$ the solute is separated as a precipitate (which can still fix some amount of water).
382 Some solute however will remain in solution, although with a much smaller concentration. It is
383 worth noting that B depends on pH and, in the case of proteins and charged amphoteric compounds,
384 reaches a minimum (*i.e.*, its maximum negative value) at the isoelectric point of the solute (where
385 usually precipitation attains a maximum rate).

386 The formation of coexisting aqueous phases within a given system occurs because of the
387 presence of thermodynamically incompatible water soluble macromolecules. In simple words, it
388 may be said that different macromolecules compete with one another for the available water and
389 tend to form aqueous phases of their own (Figure 10): each phase is largely enriched in a single
390 macromolecule, while the concentration of the other polymers is vanishingly small [Tolstoguzov,
391 2003]. Solute-solute electrostatic and/or hindrance interactions can produce additional repulsion and
392 attraction effects that can sustain the phase separation. Water activity has the same value in every
393 separated aqueous phase. To explain the process with the equation (11), it may be said that a
394 polymer solute in a given aqueous phase has a strongly negative B because of its interactions with
395 any dislike macromolecule; therefore only one polymer is allowed to remain within that phase. The
396 excluded solutes do not necessarily precipitate: instead they form other aqueous phases where they
397 can prevail. One example is the aqueous solution of a protein and a carbohydrate [Grinberg &
398 Tolstoguzov, 1997], like gelatin and dextran, that splits in two aqueous phases which are rich in
399 protein and in carbohydrate, respectively. One of these phases is finely (2-5 micrometer droplets)
400 dispersed in the other that appears like a continuous matrix.

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

403 Fundamental studies on this subject considered polymer solutions with low (10^{-3} M) and
404 very low (less than 10^{-4} M) solute concentrations. Since B of biopolymers usually is rather small,
405 large changes of a_w produce minor effects on B . That is why most of these studies directly concern
406 the solute properties and the solute-solute interactions (mainly through spectroscopic and NMR
407 investigations). This is not the case of real biological systems and food products, where c can be

408 rather large. Small changes of the moisture content can imply a large drop a_w (remind the shape of
409 sorption isotherms for low water/dry matter mass ratios) and therefore produce large effect on the
410 overall structure and organization of the system, especially when some shearing stress is applied
411 [Tolstoguzov, 2003].

412

413 **DEHYDRATION OF FOOD SYSTEMS**

414

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

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

424 To characterize the system undergoing the treatment one does not need to define the whole
425 desorption trend. It is instead necessary to assess the correlation between a_w and moisture content
426 in a limited range, namely, between the a_w of the starting fruit pulp and the a_w of the sugar syrup
427 used in the osmo-dehydration treatment. In these samples the water mobility is large enough to
428 attain the thermodynamic equilibrium of water partition between intra- and extra-cellular
429 environment. This allows the assumption that $a_w = RH \cdot 10^{-2}$. The Knudsen thermogravimetry can be
430 used to mimic in a continuous way the dehydration process [Pani & Schiraldi, 2010]. If no damage
431 of the cell membranes has occurred, the water migration from the fruit cells toward the surrounding
432 hypertonic sugar syrup requires 2 – 4 hours. One needs to define three desorption trends relevant to:
433 (I) the non-treated fruit pulp, (II) the sugar syrup used in the process and (III) a partially osmo-
434 dehydrated fruit pulp (Figure 12).

435 The trend (I) reflects the release of water from the fruit cells and therefore represents the
436 state of water in the cytoplasmic environment. The trend (III) lays between the other two, since the
437 water released from the fruit cells into the extra-cellular environment dilutes the syrup coming from
438 the bath where the fruit has been poured for the treatment. This means that trend (III) reflects the
439 state of water in the extra-cellular regions. At any dehydration level, water activity must be the
440 same in the three phases. Looking at the dehydration trends (Figure 13), the gap between trends (I)
441 and (II) and the position of the trend (III) in the middle can be used to predict the mass proportion

442 between intra- and extra-cellular phases in corresponding partially osmo-dehydrated sample [Pani
443 & Schiraldi, 2010]. On the other hand, if one considers a given overall moisture/dry matter mass
444 ratio, the water activity of a heat dried sample, which too is represented by the trend (I), is always
445 lower than that of a osmo-dehydrated fruit pulp.

446

447 **CONCLUDING REMARKS**

448

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

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

463

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514 **Appendix**

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

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

521

$$522 \quad U_w \approx u_w \cdot \Delta\mu_w = u_w \cdot RT \ln \frac{a_{w,i}}{a_{w,s}} \quad (A1)$$

523

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

528

$$529 \quad U_w = dm/dt \quad (A2)$$

530

531 and

532

$$533 \quad dm/dt = u_w [RT \ln (a_{w,i} / a_{w,s})] \quad (A3)$$

534

535 Equation (A3) allows one to recognize that

536

$$537 \quad \lim_{u_w \rightarrow 0} (dm/dt) = 0 \quad (A4)$$

538

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

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

547

$$548 \quad u_W \cdot RT \ln \frac{a_{W,i}}{a_{W,s}} = \frac{p_W^{\text{app}}}{K} \quad (\text{A5})$$

549

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

552

$$553 \quad a_W^{\text{app}} = \frac{p_W^{\text{app}}}{p_W^*} = \frac{Ku_W}{p_W^*} \cdot RT \ln \frac{a_{W,i}}{a_{W,s}} \quad (\text{A6})$$

554

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

557

$$558 \quad a_W^{\text{app}} \propto u_W \quad (\text{A7})$$

559

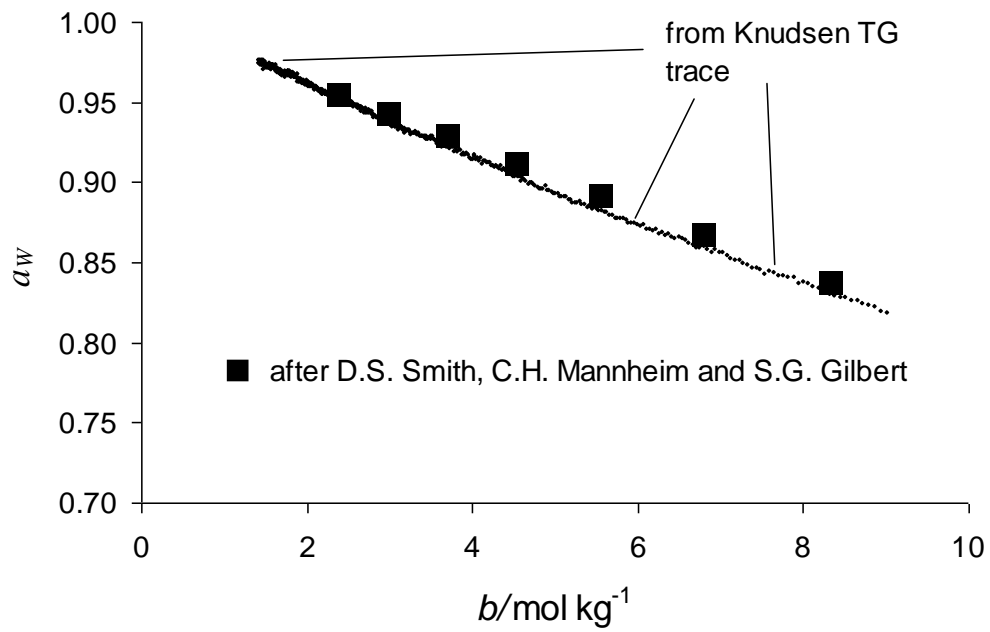
560 Since the mobility of water is inversely proportional to the viscosity, η_W , experienced by water
561 molecules that migrate from the core to the surface of the system, one may use equation (A7) to
562 compare the apparent water activity, drawn from direct or indirect measures of p_W , to the “true”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

$$564 \quad a_W = a_W^{\text{app}} (\eta_W / \eta_W^*) \quad (\text{A8})$$

565

566

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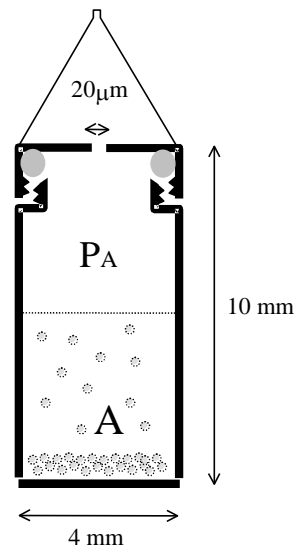
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569 *Figure 1. Water activity of aqueous glucose at various glucose molalities. The continuous line*
570 *directly comes from a Knudsen TG run, while the dots are the data reported in reference [Smith et*
571 *al., 1981].*

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Figure 2. A schematic view of a Knudsen cell hanging in the thermo-balance.

578

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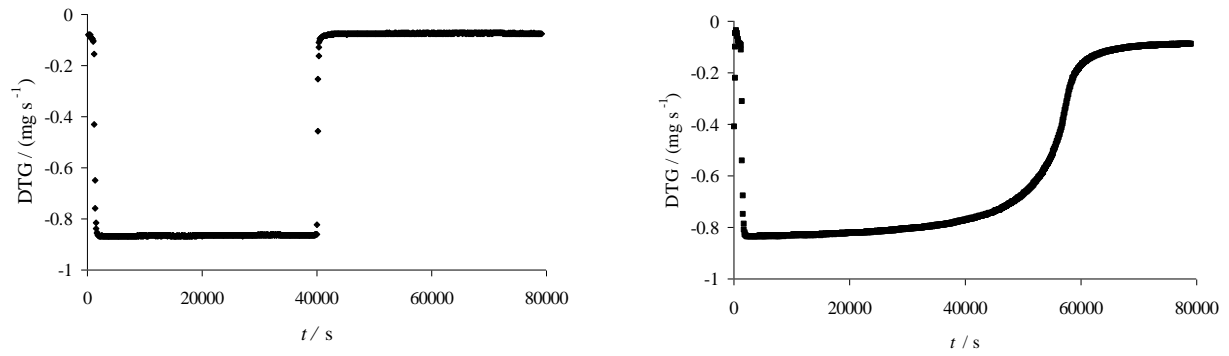


Figure 3. Knudsen isothermal desorption traces reported as DTG (time derivative of TG): (left) from pure water (a similar trend is observed for a saturated salt solution), (right) from a non saturated sample that modifies its composition.

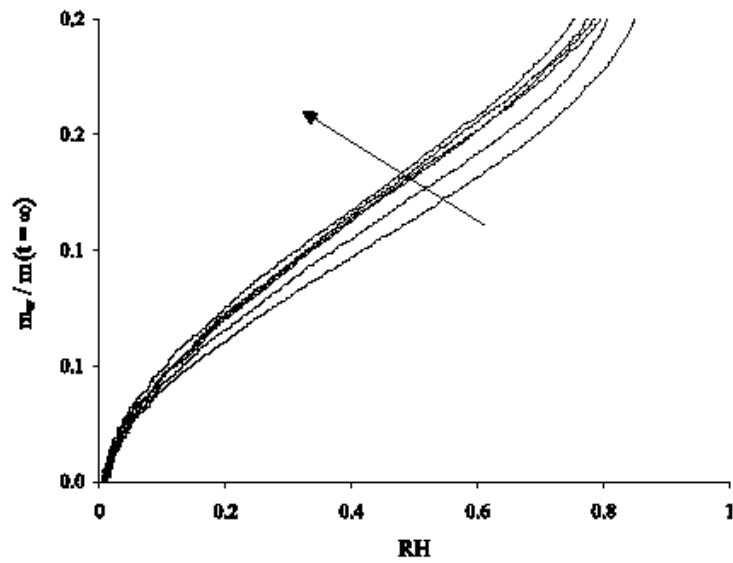


Figure 4. Desorption isotherms determined with Knudsen thermo-gravimetry for bread crumb samples of different age (from [Fessas & Schiraldi, 2005]).

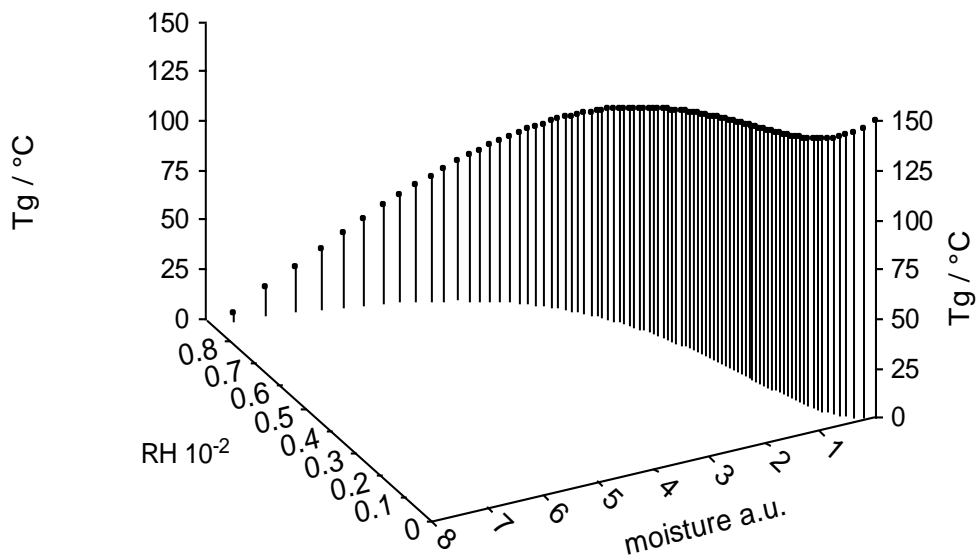


Figure 5. Schematic representation of the trend of RH (at room temperature) and that of the glass transition temperature versus the moisture content (in arbitrary units).

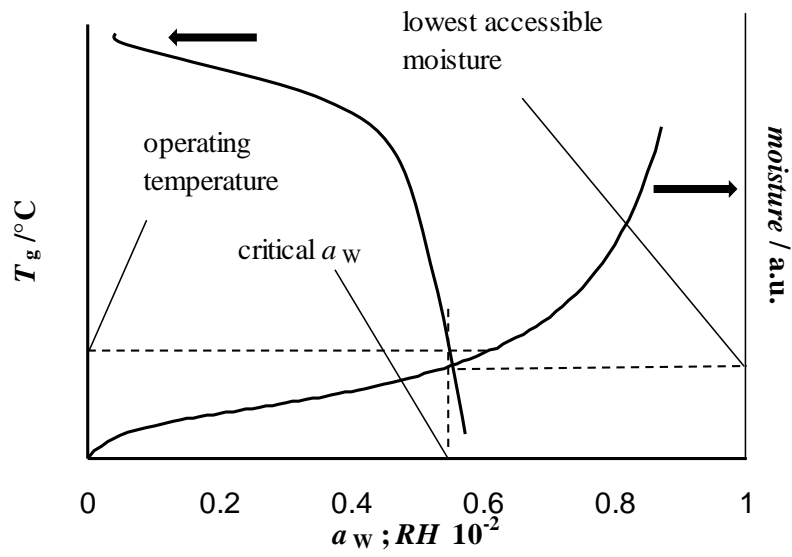


Figure 6. Identification of the critical water activity that defines the reliability limit of the isothermal sorption curve.

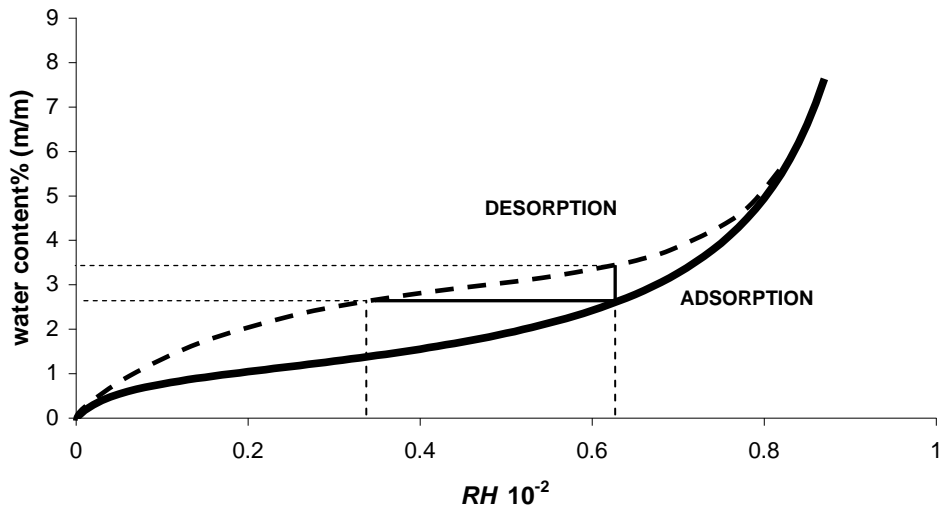


Figure 7. Hysteresis between adsorption and desorption isothermal trends

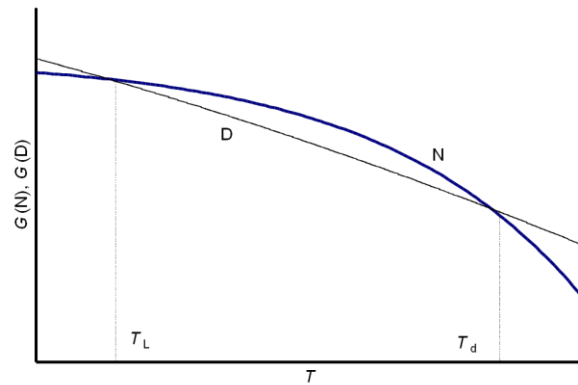


Figure 8. Gibbs function of native (N) and denatured (D) conformation of a protein in aqueous solution. The intersection points define the thermal and cold denaturation temperature, T_d and T_L .

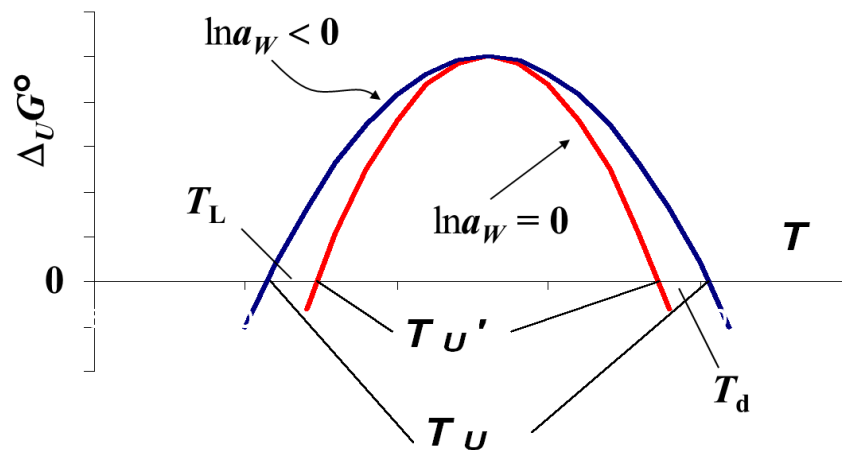


Figure 9. The unfolding temperature, T_U , of a protein in aqueous media changes with water activity, a_w . The two state model implies unfolding at two temperatures, T_d and T_L , that define the stability range of the native conformation. On decreasing a_w these limits move apart widening the stability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

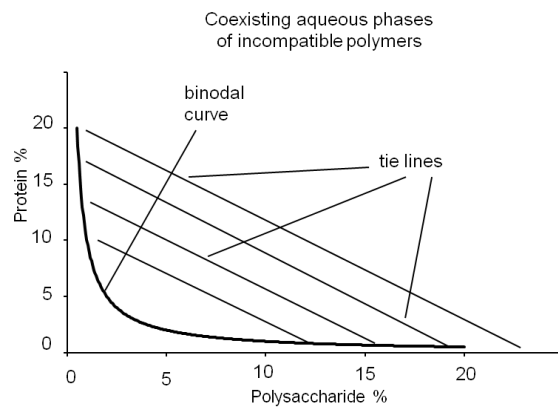


Figure 10. Phase diagram of two aqueous thermodynamically incompatible polymers. Above the binodal curve the system is split in two aqueous phase the composition of which is determined by the intercepts of the tie-lines with the binodal curve.

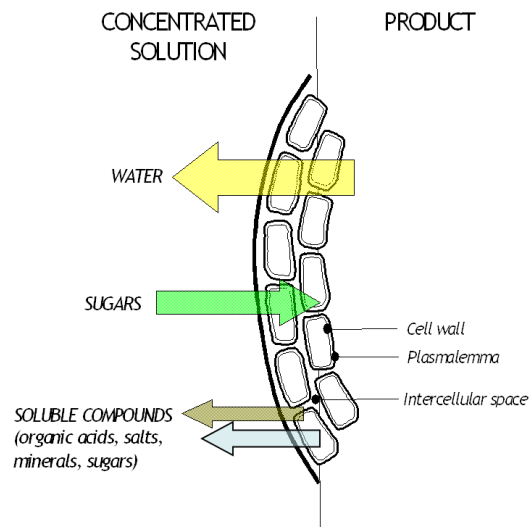


Figure 11. Matter flows involved in a osmo-dehydration treatment of fruit pulp.

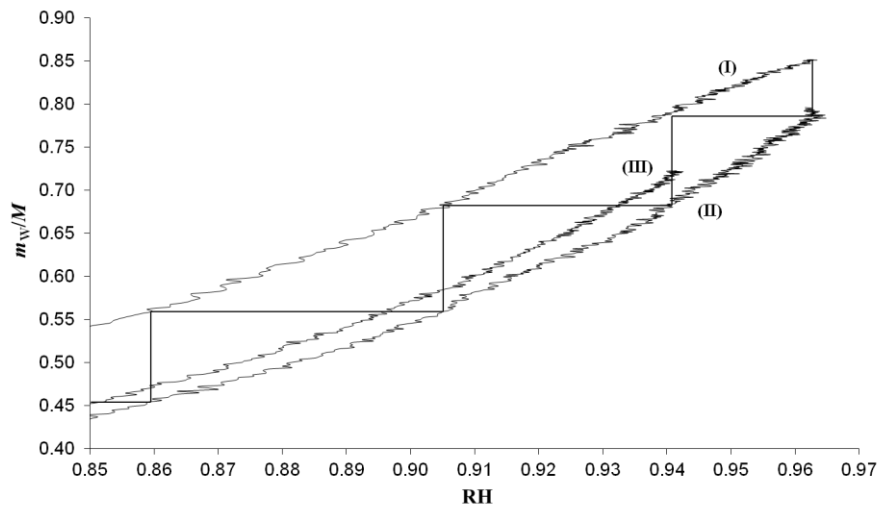


Figure 12. Knudsen desorption trend of apple pulp (I), hypertonic glucose syrup (II) and partially osmo-dehydrated apple pulp (III).