Handbook of Molecular Gastronomy
Scientific Foundations, Educational Practices, and Culinary Applications
Róisín M. Burke, Alan L. Kelly, Christophe Lavelle, Hervé This vo Kientza

Bioactivity and Its Measurement

Publication details
Hervé This vo Kientza
Published online on: 09 Jun 2021

How to cite :- Hervé This vo Kientza. 09 Jun 2021, Bioactivity and Its Measurement from: Handbook of Molecular Gastronomy, Scientific Foundations, Educational Practices, and Culinary Applications CRC Press
Accessed on: 11 Oct 2023

PLEASE SCROLL DOWN FOR DOCUMENT

Full terms and conditions of use: https://www.routledgehandbooks.com/legal-notices/terms
This Document PDF may be used for research, teaching and private study purposes. Any substantial or systematic reproductions, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.
The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The publisher shall not be liable for an loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.
Bioactivity and Its Measurement

Hervé This vo Kientza1, 2
1 Group of Molecular Gastronomy, INRAE-AgroParisTech International Centre for Molecular Gastronomy, F-75005, Paris, France
2 UMR 0782 SayFood, AgroParisTech, INRAE, Université Paris-Saclay, 91300 Massy, France

For studies in molecular and physical gastronomy, quantitative descriptions of the exchange of compounds between the various compartments that make up food, and also between these and the human body (from pans to intestines), are needed. In this chapter, the concepts of “absolute compound release” and “instant compound release” are introduced, and the concept of “matrix effect” is derived from them.

Foods are physical and chemical systems (Figure 12.1), but they are special ones, because they have to be adapted to human consumption. The interest in their properties (nutritional, sensory, etc.) does not lie solely in their microstructure or in their chemical composition but also in their interactions with the human body. As described in other chapters of this book, dishes are formulated products, often colloidal in nature (This, 2005; Dickinson, 2006). The fact that some of their compounds can be released and interact with biological receptors (mouth, stomach, etc.) has been called “bioactivity” (This, 2016), but this property needed quantification. In this chapter, we address this before looking at applications.

For this discussion, let us define as “bioactive” any compound that can interact with a biological receptor. In some cases, physical binding is needed to trigger physiological effects (e.g., olfaction, taste perception, or trigeminal effects), but for vision, the effect is indirect, and for receptors inside tissues, a transfer into the blood system is needed, sometimes after modifications during digestion.

In order to characterize quantitatively the property of bioactivity, parameters have to be introduced. There has been some confusion over definitions. Two terms – bioavailability and bioaccessibility – have been used with different meanings in different scientific or technological communities. For example, in 1999, Shargel and Yu defined the bioavailability of nutrients as a quantity measured in the blood plasma of humans (in vivo assay), so factors such as individual variability, physiological state, dose, and presence of other meal components come into play (Shargel and Yu, 1999). Although the whole of a nutrient is potentially bioaccessible, in reality, almost no nutrient is totally converted during digestion into a potentially absorbable form.

In 2000, Oomen et al. observed that “the concept of bioavailability is interpreted in the literature in various ways” (Oomen et al., 2003). According to the general interpretation in pharmacology, oral bioavailability is presently defined as “the fraction of an orally administered dose that reaches the systemic circulation”.

In 2002, however, the Food and Drug Administration (FDA) proposed to define bioavailability as

the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

(Shargel and Yu, 1999; Shi and Le Maguer, 2000; FDA, 2002)

In particular, “absolute bioavailability” was defined as the quantity and rate that characterize the transfer of the drug in blood, and “relative bioavailability” as a measurement of the quantity and speed that characterize the transfer of a drug when many forms are compared with a reference form, for example.

This definition was proposed to apply also to active substances (nutrients) present in foods; in nutritional science, it is the proportion of a nutrient that is absorbed by the body.

\[f(G, O, S, W) \rightarrow f'(G, O, S, W)\]

\[\text{Culinary transformation}\]

Release of Bioactive

Release of Bioactive

FIGURE 12.1 One important question for molecular and physical gastronomy is to study how compounds are differently released by food ingredients when food systems (as described by the DSF formalism) are processed, starting from a system \(f(G, O, S, W)\) and producing a system \(f'(G, O, S, W)\).
In 2002, Hedren et al. were using the term “bioaccessibility” as the amount of an ingested nutrient that is available for absorption in the gut after digestion. Thus, it is not equivalent to speak of bioavailability or bioaccessibility. If the amount of recovered nutrient after digestion is of relevance, then the term to use is bioaccessibility. On the other hand, bioavailability of nutrients is usually measured in the blood plasma of humans (in vivo assay).

(Faulks and Southon, 2005)

Although all of a nutrient is potentially bioaccessible, in reality, almost no nutrient is totally converted during digestion into a potentially absorbable form. In almost every case, the bioaccessibility and bioavailability of a nutrient are governed by the physical properties of the food matrix, which affect the efficiency of the physical, enzymatic, and chemical digestion processes (Boyer and Liu, 2004).

Confronted with different definitions, the International Union of Pure and Applied Chemistry (IUPAC) proposed in 2004 to define bioavailability as the “extent of absorption of a substance by a living organism compared to a standard system” (IUPAC, 2004). For toxico- or pharmacokinetics, a quantitative definition was given as the ratio of the systemic exposure from extravascular (ev) exposure to that following intravenous (iv) exposure as described by the equation:

\[ F = \frac{A_{ev}D_{iv}}{B_{ev}D_{iv}} \]  

(12.1)

where \( F \) (fraction of dose absorbed) is a measure of the bioavailability, \( A \) and \( B \) are the areas under the (plasma) concentration time curve following extravascular and intravenous administration, respectively, and \( D_{ev} \) and \( D_{iv} \) are the administered extravascular and intravenous doses.

However, the various authors and the various communities did not always stick to this definition (Gregory et al., 2005). In 2007, bioaccessibility was defined (IUPAC, 2007) as the “potential for a substance to come in contact with a living organism and then interact with it. This may lead to absorption”. A note was added as follows:

A substance trapped inside an insoluble particle is not bio-accessible although substances on the surface of the same particle are accessible and may also be bio-available. Bioaccessibility, like bio-availability, is a function of both chemical speciation and biological properties. Even surface-bound substances may not be accessible to organisms which require the substances to be in solution.

In 2012, it was observed that the release of compounds by food ingredients during cooking, or by food in the mouth or in the digestive tract, can be described by measuring the net disappearance of the compounds from the food items, and quantitative parameters called “absolute bioactivity” and “dynamic bioactivity” were introduced (This, 2012). However, in spite of very clear definitions of these “bioactivities”, confusion between them and the two words “bioavailability” and “bioaccessibility”, as well as the fact that a compound can have a bioactivity (i.e., an action on the body), led to a change, which will be proposed later: we shall see that the new proposed terminology is “compound release”.

This proposal should be helpful, because as said, today various scientific communities are still using old definitions, so it is sometimes difficult for them to exchange information. In 2015, Collins et al. proposed the view shown in Figure 12.2, adding that there are a number of definitions of bioaccessibility, these can be particularly confusing because they can relate to both human ingestion and microbial degradation. We use the following definition: the maximal amount of contaminant released from the test matrix in a synthetic gastrointestinal system. This fraction represents the maximum amount of a

![FIGURE 12.2](image_url)
contaminant that is available for absorption within the human gastrointestinal tract.

**Compound Release (CR)**

With the proposed quantitative parameters associated with “compound release”, we do not want to focus exclusively on nutritional properties, or flavor properties, or toxicological effects; more generally, we want to describe the exchange of any compound between a (possibly food) system and its environment, in particular during cooking; such activities are often based on the thermal treatment of plant or animal tissues in an aqueous solution. As molecular and physical gastronomy seeks to characterize and understand the effect on how food exchanges compounds with the body, we define the first notion using quantitative indexes such as “absolute compound release” ($ACR_p$) for the description of the total content of bioactive compounds, and “dynamic compound release” ($DCR$) for the time-course release of compounds from food systems.

Let us start from a food system $F$, containing a mass $m$ of a compound $C$. Concerning “absolute compound release”, a difference is introduced between “potential absolute compound release” ($ACR_p$) and “actual absolute compound release” ($ACR_a$), because the presence of a compound in a matrix is not a guarantee that all the molecules of the compound will be able to leave the matrix (and possibly interact with receptors). We propose to define “potential absolute compound release” as the possibility to bind to receptors and to measure this property as the mass of the compound $C$ present in the food matrix or at its surface. On the other hand, “actual absolute compound release” is the real quantity (mass) of the compound that can be released. Of course,

$$0 < ACR_a < ACR_p$$

These definitions go along with a definition of an “absolute matrix effect”, $E$, defined in such a way that there is no matrix effect ($E = 0$) when the compound is entirely released ($ACR_p = ACR_a$), and a maximum matrix effect ($E = 1$) when the matrix completely traps the compound ($ACR_a = 0$) (Chemistry International, 2009; Engel et al., 2001; Kördel et al., 2009). A simple definition can be (if the matrix effect is chosen as a dimensionless parameter):

$$E = \frac{ACR_p - ACR_a}{ACR_p}$$  (12.2)

In general, the food system $F$ is an object of complex shape inside an environment $M$. Let $S$ be its surface, and let $t$ be the contact time of $F$ and $M$. A mass flux (mass exchanged through the surface $S$ by time unit) can be defined algebraically as:

$$\frac{dm(t)}{dt} = \int_S j(t) \, ds$$  (12.3)

where $ds$ is a surface element through which a quantity $j(t)$ of the compound $C$ is exchanged between the food system $F$ and its environment, the integration being taken over the entire surface $S$ of $F$.

Using this definition, the released quantity of the compound $C$ is equal to:
where the integration is taken between the contact time considered 0 and +∞.

Using this definition, we can now consider a definition of “dynamic compound release” (DCR). When the matrix effect is physical in nature (no molecular modification of a compound during the transfer), a dynamic compound release can be defined for each compound being exchanged between F and M.

Let us first observe that one could calculate dynamic compound release as the variation of the released quantity as a function of time, but the new parameter would not have the same dimension as absolute compound release. This is why we proposed to define the dynamic compound release after “releasability” l(t):

\[ l(t) = \frac{dm(t)}{dt} \quad (12.5) \]

Using this function, dynamic compound release \( DCR(t) \) can be defined as:

\[ DCR(t) = \int_{0}^{t} l(t) \, dt \quad (12.6) \]

where integration is taken between 0 and t.

Using this definition, the dynamic compound release \( DCR \) for \( t \to +\infty \) is equal to the actual absolute compound release \( ACR_{\infty} \).

Whereas the dynamic compound release describes the exchange of the compounds as a function of time, the dynamic matrix effect \( e(t) \) describes the variation of the rate (because of the matrix) at which a compound is released. For the same reasons as before, the dynamic matrix effect has to be a dimensionless function of time, and a reference has to be chosen. It is proposed that the dynamic matrix effect is nil when no matrix is present, so that releasability \( l(t) \) has to be compared with releasability without a matrix (pure diffusion):

\[ e(t) = \frac{f(t) - l(t)}{f(t)} \quad (12.7) \]

where \( f(t) \) is releasability without a matrix (simple molecular diffusion in the considered environment). Of course, in order to determine this function, one has to consider an object having the same shape as the food system F (same surface), with:

\[ f(t) = \int S f(t) \, ds \quad (12.8) \]

the integration being taken over the entire surface S.

Whereas the physical matrix effect describes the molecular interaction of particular compounds with the matrix, a “chemical matrix effect” is used when molecular transformations occur between a compound and the matrix during culinary transformations (thermal treatment, i.e., “cooking”, being a particular case of such transformations). In order to define a chemical matrix effect, one considers a compound that is modified during the F–M interaction. Chemical processes reduce the released quantity of this compound. The absolute matrix effect \( E \) describes both the chemical as well as the physical matrix effects, but the dynamic matrix effect only indicates an influence of the matrix on the overall release of a compound without taking into account the molecular transformations. In order to introduce a “chemical dynamic matrix effect”, one has to make assumptions about the independence of the compounds and their reaction products. When chemical transformations occur, the release of a compound is reduced (releasibility \( l(t) \) instead of \( l(t) \)) by processes that make a quantity \( r(t) \) (per time unit) disappear. One can write:

\[ l(t) = l(t) - r(t) \quad (12.9) \]

Here again, the dynamic chemical matrix effect \( e_c(t) \) can be introduced as a dimensionless function:

\[ e_c(t) = \frac{r(t)}{l(t)} \quad (12.10) \]

Using this definition, the chemical dynamic matrix effect is nil when there is no chemical transformation at any time, and equal to 1 when the bioactive compound is entirely transformed within the matrix (\( r(t) = l(t) \)). Depending on the experimental situation, the other definition can be used:

\[ e_c(t) = \frac{l(t) - l'(t)}{l(t)} \quad (12.11) \]

### In situ Quantitative NMR

How can the transfers of compounds in matrices be followed and all these parameters numerically estimated? The analytical method called in situ quantitative nuclear magnetic resonance (iq q NMR) was proposed for this purpose, based on the fact that gels are colloidal systems that enclose a liquid component. This method was first studied for the analysis of saccharides, amino acids, and organic acids of plant tissues (This et al., 2010; Weberskirch et al., 2011), and compared with the classic method for the determination of the saccharide content of plant tissues devised by Davis et al. (2007) as a modification of the method set up by O’Donoghue et al. (2004) and others (Viola and Davies, 1992; Kahane et al., 2001). In the “modified O’Donoghue” method, plant tissues are lyophilized, heated under reflux in a mixture of methanol and water (62.5: 37.5, w:w) for 15 min at 55 °C; after filtration, solvent evaporation, and lyophilization, the resulting product is analysed using various analytical techniques, such as quantitative proton nuclear magnetic resonance spectroscopy (q 1H NMR) (Cazor et al., 2006; Tardieu et al., 2009). However, analyses done by this modified O’Donoghue method are long and destructive and require manipulations with toxic solvents.
As metabolites from plant tissues, including saccharides, are supposed to be dissolved in an aqueous cytosolic environment (Clerg, 1984), it was important to determine how much of them could be detected using is $^1$H NMR of whole tissues.

Our studies (Weberskirch et al., 2011) were performed using carrot (Daucus carota L.) roots, in which the mean water content is about 88% of fresh weight (Nonnecke, 1989). In these tissues, as in most plant tissues, the cytoplasm of plant cells is a gelled system (Turner, 1981), where metabolites and ions are in an aqueous, liquid environment that can be described by the DSF (see the chapter on this) formula $D_0(W)/D_3(S)$. In the cytoplasm, the cytoskeleton forms a network, including an aqueous solution of metabolites (cytosol) in which organelles are dispersed. Moreover, a free aqueous solution makes up the sap filling the vessels, called xylem and phloem; saccharides are dissolved in the elaborated sap (Campbell, 1995).

The is $^1$H NMR method was compared with extraction followed by quantitative NMR spectroscopy of extracts (q NMR): non-treated samples of plant tissues were introduced into a 5 mm glass NMR tube with enough $D_2O$ for locking (in practice, about 0.05 g). Experiments validated the assumption that plant tissues could be directly studied by the application of liquid q $^1$H NMR spectroscopy to whole, non-treated tissues, in spite of a large quantity of ordinary (i.e., non-deuterated) water being present in the tissues. In particular, even if the baseline is sometimes deformed in some way, saccharides can be easily determined in the aqueous environment of cells and of conducting tissues (for the latest results, a 1/1000 precision was obtained).

NMR spectra obtained by direct determination have the same general appearance as NMR spectra obtained with solutions of saccharides, including solutions made using the modified O’Donoghue method (O’Donoghue et al., 2004). Moreover, with both MOD/q $^1$H NMR and is $^1$H NMR methods, a quantification of the major saccharides (D-glucose, D-fructose, and sucrose) in carrots was possible with minimal mathematical treatment. The main resonance of spectra obtained by both methods was due to water; this was surrounded by resonances of various metabolites, including primarily saccharides and amino acids. Saccharides are not the only metabolites that can be quantitatively determined; in particular, many resonances associated with amino acids can also be studied following some mathematical treatment. One difference between the spectra in the two methods was the intensity of the resonances; using is $^1$H NMR, the resonances are smaller than with the MOD/q $^1$H NMR method (but still more than 10 times the noise level), because the mass of plant tissue used for the direct determination (0.2 g) is about 50 times lower than the mass of fresh tissue used in the MOD/q $^1$H NMR method (7–10 g). Of course, the water resonance is huge with is $^1$H NMR, but this does not prevent the determination of the important metabolites (using signal decomposition). As the characteristic resonance of D-glucose at a chemical shift of 5.24 ppm was partly covered by the water resonance, the other characteristic resonance at 5.25 ppm was used for the direct determination (in any case, resonance decomposition can be used even when a signal merges into another, bigger signal). Figure 12.4 shows the general shape of the spectra.

**FIGURE 12.4** An is $^1$H NMR spectrum for a carrot (Daucus carota L.) root sample.

(Cazor et al., 2006).
In some spectra obtained by the in situ method, the multiplicity of the sucrose and D-fructose resonances was not observed, and some analyses gave irregular spectra, where the singlet of 3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid, sodium salt (TSP, used as a chemical shift reference and also for quantitative determination) was split into a doublet. These poor spectra were generally associated with a magnetic field heterogeneity that could not be corrected easily. As the “shim” gives information about the homogeneity of the magnetic field in the sampled sample, it is an important factor for the quality of the spectra. The solution we found to overcome this irregularity was to partially dry the tissues before analysis. Thus, both the water resonance is minimized and the D-glucose resonance at 5.24 ppm (H₁α) is more visible in the spectra (however, the D-glucose resonance at 3.25 ppm was used for quantification because it gives better results).

In general, the direct, in situ NMR determination gives a higher saccharide content than the modified O’Donoghue extraction/q NMR method. However, this trend is statistically significant only for D-glucose and sucrose (one-way analysis of variance test, significance level of 1%). Of course, the is q NMR method is much faster and easier than the method using extraction and NMR analysis: whereas sample preparation takes six days for the extraction and NMR analysis (because of double lyophilization), only a few hours are needed with the is q NMR method. Moreover, methanol is not used in the is q method.

Classification of Bioactivities

With a rational way to study bioactivity and methods for the determination of compound release from colloidal systems, let us now consider theoretically what the matrix effect could be. Indeed, such an effect is due both to structure and to the binding of compounds to molecular details of the matrices. Such interactions can be ranked in order of energy (Atkins, 1990).

At the low-energy end of the interaction energy spectrum, van der Waals interactions can limit the autodiffusion coefficient of compounds; this is the case for most solutions. At higher energies, hydrophobic forces or hydrogen bonds can play a role. At even higher energies, we can envision the effect of disulfide bridges, with the ability to rearrange, as in dynagels (This, 2016).

At the highest end of the energy scale, covalent and electrostatic bonds are important.

One important question is whether the matrix effect in complex disperse systems can or cannot be described as a result of elementary interactions between the various phases. This question was addressed first by analysing the processing of “vegetable stocks”, i.e., aqueous solutions obtained by thermal processing of plant tissues in water (Cazor et al., 2006). In spite of extensive use of carrot stock in homes, in restaurants, and in the food industry, very little was known about metabolites found in the stock and the time evolution of their concentration during the thermal processing. The release of saccharides from carrot root samples thermally processed in water was studied for various reasons (Varoquaux et al., 1986), such as identification of stock composition, loss of vitamins, and nitrate release in food products. The mechanism of saccharide release was also studied. Solutes are present either in phloem sap or in intra- and intercellular spaces (Stapley et al., 1995). Hence, saccharides can be released from any of these compartments.

In one study (before is q NMR was set up), q¹H NMR was used for the quantitative determination of saccharides in carrot stocks prepared at three temperatures: 50, 75, and 100 °C. Using the protocol of Cazor et al. (2006), carrot roots were peeled, and the bottom and top parts were removed. Then, the samples were cut into cylinders of a specific size and heated in demineralized water at a determined temperature. The aqueous solution samples were centrifuged, filtrated, double freeze-dried, and dissolved in D₂O with pH adjustment to 7 (using solutions of DCl). For the NMR analysis, an Ultra Shield Bruker 500 MHz spectrometer was used at T = 21 °C (±0.1 °C); 64 scans of 65 k, spectral width 6 kHz, Δq 5.3 s, ΔI 25 s.

The main compounds found in carrot stocks were saccharides, amino acids, and organic acids. Three regions were identified, corresponding to the amino acid region (0–3 ppm), the saccharide region (3–6 ppm), and the phenolic region (6–10 ppm). Each major metabolite of the different extracts was identified after resonance assignment using ¹H NMR spectra from pure compounds associated with comparison of published data (Fan, 1996; Le Gall et al., 2003). Twenty-one metabolites were identified on each spectrum, including 3 saccharides, 11 amino acids, 7 organic acids, and other compounds.

The thermal processing caused significant enrichment of the water environment in saccharides (Figure 12.4). For all three saccharides studied, extraction was less effective at 50 °C than at the two other temperatures (75 °C and 100 °C). As the optimal temperature for carrot pectin methyl esterase (PME) activity is in the range 40–60 °C for pH values between 4.5 and 7 (Ly-Nguyen et al., 2002), we proposed to explain this difference by enzymatic activity; at 50 °C, cell membranes remain intact in spite of some protein denaturation, and loss of nutrients occurs mainly due to osmosis, capillarity, and diffusion (Garrote et al., 1984; Oliveira, 1988). Carrot PME activity is responsible for pectin linking through calcium cations, and diffusion through a cell wall is more difficult than diffusion through a cell wall with hydrolysed pectic material in the high-temperature regime. Alzamora et al. (1985) showed that, in order to minimize leaching losses of vitamins during water blanching of peas, it is preferable to have a high temperature in “static” water and the biggest peas possible. On the contrary, to maximize saccharide recovery in a water solution, higher temperatures than 60 °C are to be chosen.

To explain the increase in D-glucose and D-fructose concentration at 100 °C, associated with a decrease in sucrose level at the same temperature (Figure 12.5), sucrose hydrolysis was proposed. In theory, this assumption could be checked by correlating the sucrose with the D-glucose+D-fructose concentrations. However, glycation reactions occurring between reducing saccharides and amino acids also have to be taken into account.
Modelling Saccharides

The quantitative study of the time-course evolution of saccharide concentration in carrot or onion stocks led us to some assumptions about the mechanisms of this evolution. In Figure 12.6, the left rectangle stands for the inside of the plant tissue. In this diagram, the letters S, G, F, and H represent sucrose, D-glucose, D-fructose, and 5-hydroxymethylfurfural, respectively; the second letter (c or b) indicates the carrot tissue or the stock.

Given measured initial conditions, a differential system, assuming linearity, can lead, with simplifying assumptions, to a solution such as that in Figure 12.7. From the initial equations, sucrose, D-glucose, and D-fructose have to decrease in the plant tissue and increase in the stock. Sucrose in the stock should, however, decrease because of hydrolysis. Although fitting experimental data with the functions obtained in solving general cases can give constants for transfers and various chemical processes occurring during thermal treatment, it is unlikely, due to uncertainties in experimental data, that any new scientific result will be obtained using this strategy. On the other hand, using a different model provides other information, for example, where tissues are considered to be made of channels (xylem and phloem) and parenchymous tissues, such as in Figure 12.8 (model based on microscopic pictures such as Figure 12.9).

Such pictures (in this case, an onion bulb sample soaked in methylene blue, 3,7-bis(dimethylamino)-phenothiazin-5-iium chloride) indicate that saccharides and other solutes can migrate
from some aqueous solutions toward the inside of plant tissue. If this phenomenon were due to molecular diffusion, it could also occur in the opposite way, from plant tissues toward an aqueous environment, such as in many culinary processes.

In order to model the extraction of water-soluble compounds from onion bulb tissues in an aqueous environment, these compounds have to be identified, and their concentration has to be estimated in the aqueous solution at different times. The phenomenon of extraction of solutes from plant tissues to the soaking solution was investigated experimentally, and the data were studied analytically for bulbs of onions (*Allium cepa* L.). With Marcia France and Audrey Tardieu (Tardieu et al., 2011), we explored the time-course extraction features, considering that the solutes could be located either in channels or in parenchyma cells; short and long times of extraction were studied. First, only two compartments were considered: “channels” and “parenchyma cells”. Two main limit profiles for metabolites can be considered: quantity in parenchyma cells >> quantity in channels; or quantity in channels >> quantity in parenchyma cells. For each case, there are two possibilities: release from parenchyma cells faster than release from channels, or release from channels faster than from parenchyma cells. Another case could be quantity in parenchyma cells ~ quantity in channels, with the two transfer rates being equal or different (Figure 12.10).

Fitting curves of the dry matter against time measured during experiments indicates that approximately the same quantity of saccharides is extracted from parenchyma cells and from channels, with a large difference in transfer rate (0.048 for the former, 0.876 for the latter; arbitrary units). At this point, we do not know which compartment releases bioactive compounds faster, but, if pure molecular diffusion occurs from channels, as was observed when plant tissues were soaked in methylene blue, it is likely that this compartment is responsible for faster exchanges than the other.

Of course, these studies are far from finished, in particular because one can see from Figure 12.10 that methylene blue migrating toward the inside of plant tissues diffuses not only in the channels but also in the surrounding tissues. The question of measuring the fluxes and the kinetic parameters associated

---

**Figure 12.7** Modelling changes in saccharide levels during carrot stock processing. Here the variations are calculated for D-glucose (G), D-fructose (F), and sucrose (S). The abscissa scale is for the time, and the ordinates are arbitrary units of saccharide quantities.

**Figure 12.8** A model of plant tissues with various compartments allowing saccharide exchange.

**Figure 12.9** An onion (*Allium cepa* L.) bulb sample soaked in methylene blue shows some colourant inside channels and very little colourant inside plant cells. The diffusion of the colourant inside the plant tissues indicates that the compounds from the elaborated sap of the tissue could migrate in the reverse direction, toward the aqueous environment, during cooking. However, the presence of blue colour around the channels shows that diffusion from sap to the aqueous environment is not the sole mechanism responsible for stock making.
FIGURE 12.10 One particular behaviour that can be obtained for the model of Figure 12.8 restricted to two compartments (“cells” and “channels”). Curve 1 corresponds to the compartment richest in metabolites; Curve 2 to the compartment with the lowest concentration in metabolites; and Curve 3 to the sum of the two phenomena.

with them remains an important goal for understanding the mechanisms of a simple culinary process such as a stock.

REFERENCES
IUPAC. 2004, Glossary of terms used in toxicokinetics (IUPAC Recommendations 2003), PAC. 76, 1033–1040.

Tardieu A, France MB, This H. 2011. NMR Determination of a Model of Solute Release from Plant Tissues in an Aqueous Environment, Fruit&Veg Processing, Avignon, France (to be published).


