



1 Calorimetry and thermal analysis in food science: an updated review

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5 Abstract

AQ1 Food science is a domain of life science. Applications of thermal analysis and calorimetry (TAC) to food products deal
7 with many investigation targets spanning from the characterization of the systems at molecular and supramolecular level to
AQ2 the description of the microbial metabolism. Food products are multi-phase and multi-component metastable systems
AQ3 where several processes can occur simultaneously during the preparation process and the shelf life. One therefore has to
10 disentangle various contributions to the overall instrumental outputs, using appropriate data treatments and kinetic models,
11 and/or results from other experimental approaches. The paper reports an updated survey of TAC applications to food
12 **AQ4** products through specific examples of data treatments.

13

14 Introduction

15 The first European paper devoted to thermal analysis and
16 calorimetry (TAC) applied to food products and processes
17 appeared in 1990 [1], followed by wider presentations in
18 1994 [2] and 1999 [3]. In that decade, food science actually
19 became a special domain of TAC application to life science
20 (Scheme 1), including many appealing subjects as investi-
21 gation targets, since food products are multi-component
22 and multi-phase metastable systems that host living
23 microbial cells.

24 As for the molecular and, above all, supramolecular
25 aspects, food science actually is a branch of polymer sci-
26 ence [4] in as much as natural polymers govern the overall
27 behavior of most food products. Food polymers are indeed
28 responsible for phase separations [5], which determine the
29 extension of the interphase regions where most of the
30 chemical reactions take place. Food polymers directly
31 affect the overall viscosity of the system and consequently
32 the diffusion rate of reactants with the ultimate limit of the
33 glass transition, below which no molecular displacement
34 can occur. This threshold mainly depends on the local
35 polymer concentration, which is not necessarily uniform
36 across the system because of: (a) the thermodynamic

incompatibility that induces phase separation and (b) the 37
large viscosity related to the presence of the polymers 38
themselves. Beside their viscosity effects, many food 39
polymers act as surfactants (proteins, nonionic polyglyc- 40
erides, propylene glycol alginate, etc.) that stabilize the 41
dispersion of various phases within a given food system 42
[6], allowing the persistence of bubbles, droplets, solid 43
particles. (A good example is the ice cream.) 44

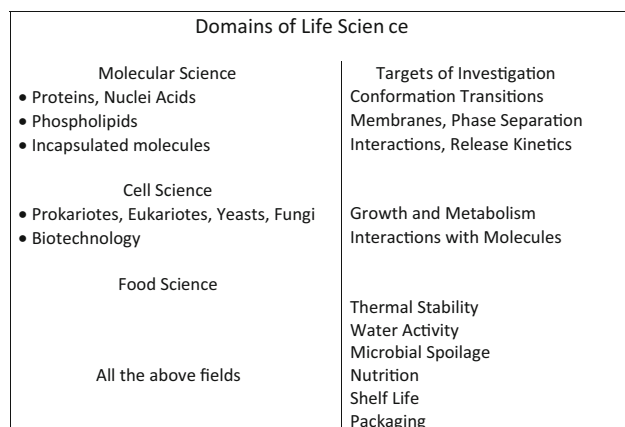
The other major component of most food systems is 45
water. Its displacements and partition between coexisting 46
phases (including dispersed phases) substantially con- 47
tribute to the physical and sensory peculiarity of a given 48
product [7]. Water enters the structure of biopolymers 49
(carbohydrates, globular proteins and gluten) [8–12], since 50
water molecules form bridges between polymer chains 51
through hydrogen bonds [11]. In spite of its large mobility, 52
water can remain trapped within a polymer network loos- 53
ing many properties of bulk water, like the ability to form 54
ice crystals or a vapor phase at the temperature where one 55
would expect it to do so. The “bound” water is indeed a 56
very popular parameter of food science as it determines the 57
practice of industrial preparations, like frozen dough for 58
bakery, ice cream, jellies, etc., and of some processes like 59
lyophilization [13], thermal [14] and osmo-dehydration 60
[15]. These aspects actually are consequences of the role of 61
water on the glass transition temperature, T_g , of wettable, 62
or water-trapping products, including powdered materials 63
(sugars, coffee, cocoa, etc.) [4, 16, 17]. 64

Because of such interactions, polymers and water make 65
the preparation of food a true endeavor, especially at the 66
industrial scale. Once the various ingredients and 67

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Scheme 1 Domains of life science to investigate with TA and calorimetry

68 respective doses are established, the preparation requires an
 69 adequate procedure to account for the phase dispersion and
 70 the overall viscosity at each step of the process. This pic-
 71 ture is even more complex because of the concomitant
 72 presence of microbes that are responsible for many
 73 chemical and physical changes that affect sensory and
 74 nutritional properties of most food products. At the
 75 household scale, the heritage of previous experiences,
 76 namely the culinary traditions, unconsciously complies
 77 with these physical and microbiological constraints,
 78 although, nowadays, a better awareness of the physics
 79 involved is at the fundament of the so-called molecular
 80 gastronomy [18] that suggests novel approaches to food
 81 preparation. Unfortunately, this is not enough for processes
 82 at the industrial scale.

83 The whole panoply of TAC (IC, DSC, TMDSC, TG,
 84 DMA, etc.) allows a thorough inspection of food systems
 85 through the quantitative determination of many properties
 86 and processes, like enthalpy and heat capacity changes,
 87 glass transition, phase separations, water activity and
 88 microbial growth [3]. The results of such investigations
 89 make the predictions of the shelf life reliable and provide a
 90 rationale for the production at the industrial scale. One just
 91 needs a representative sample of the system to investigate.
 92 According to the size of such sample, one has to select the
 93 kind of instrument to use. No pretreatment is normally
 94 required. Salads, cheese, milk, rice, chocolate, etc., can
 95 directly undergo the investigation. This possibility is of
 96 crucial importance as long as almost any food would lose
 97 its own physical and chemical properties once treated:
 98 Separation, extraction, filtration, etc., can substantially
 99 modify the food system.

100 Other techniques, like microscopy (including laser
 101 confocal microscopy), SEM, NMR, rheology, X-ray
 102 diffraction, etc., add the complementary information that

supports the interpretation of the evidences garnered 103
 through calorimetry and thermal analysis. 104

The large number of experimental approaches 105
 nonetheless leaves unresolved the true challenge for food 106
 scientists: the simultaneous occurrence of many processes 107
 that take place in the course of the investigation of a given 108
 product. The direct output of a measurement can indeed be 109
 the resultant of many coexisting phenomena and therefore 110
 require a further treatment to split them from one another. 111
 Such a treatment can be mathematical (deconvolution of 112
 traces, kinetic models, etc.) and/or implies separate 113
 experiments on model systems. 114

In the present review, some examples allow one to 115
 envisage how a food scientist can convey the interpretation 116
 of the experimental TAC data into the familiar view of 117
 chemical thermodynamics and kinetics, or traditional 118
 microbiology, selecting parameters that are useful tools for 119
 an objective description of a given food product or process. 120

Bread and starchy products 121

Bread and dough 122

Bread, one the most diffused food, is the result of baking a 123
 dough formed by mixing cereal flours containing gluten 124
 with water, salt and yeast, or chemical leaven. In spite of 125
 the macroscopic appearance, the starting kneaded dough is, 126
 at room temperature, a rather heterogeneous system [19]. It 127
 contains starch granules (the size of which ranges from 5 to 128
 50 μm), damaged starch granules, aqueous globular pro- 129
 teins and salts, dispersed non-starch polysaccharides (like 130
 arabino-xylans), partially linked glutenin and gliadin 131
 (precursors of the gluten), separated lipid fractions, yeast 132
 cells, etc. This system undergoes a substantial transfor- 133
 mation when heated during the baking treatment. The 134
 formation of the gluten network takes place just after the 135
 onset of the starch gelatinization that encompasses a wide 136
 temperature range above 50 $^{\circ}\text{C}$; the former process is 137
 exothermic, while the latter is endothermic. In the same 138
 temperature range, the globular proteins of the flour 139
 undergo unfolding (endothermic) and aggregation 140
 (exothermic). All these changes depend on the water con- 141
 tent, occurring at higher temperature for lower humidity 142
 [20]. 143

To complete the picture, one should account for the fact 144
 that the dough polymers (starch and non-starch carbohy- 145
 drates, gluten and globular proteins) compete with one 146
 another for the solvation water and are thermodynamically 147
 incompatible with one another [21], which means that they 148
 tend to form separated aqueous phases dispersed within one 149
 another. The mean size of the dispersed particles (droplets, 150
 bubbles) ranges around 5 microns, while the gluten 151

152 networks is a long-range extended structure that interpenetrates the amylopectin and amylose gels. What's more, 153 because of the size of the loaf and the standard baking conditions, a large temperature gradient exists between the 154 surface of the system, where T can attain 200 °C, and its core, where the T raises more slowly and remains below 155 100 °C (Fig. 1). This is the reason for a core-to-surface water migration [22]: Accordingly, the water content is not 156 homogeneous across the system. 157

161 In a typical baking process, water escapes from the 162 dough loaf and its final content is about half of the starting one. The main consequence is an overall hardening of the 163 system, which, however, is not uniform at all. At its surface appears the crust (that implies local pyrolysis and oxidation 164 because of the atmospheric oxygen), while its core becomes a sponge with irregular alveoli, namely the crumb. The 165 formation of alveoli starts from original nuclei of gas phase (mainly CO₂ and water vapor produced by the 166 added leaven) and continues during the baking progress with complete replacement of CO₂ with air [23]. The 167 hardening of the walls that governs the average alveolar size is the resultant of the competition for the available 168 moisture between the thermodynamic incompatible polymers mentioned above [24], among which globular proteins 169 act as surfactants at the gas/liquid interface of the bubbles. 170

178 Now let us consider what happens in a DSC investigation of a dough sample that normally does not exceed 179 10 mg. Since the DSC cell is sealed, no water loss takes place. The temperature gradient across such a sample is 180 much smaller than in a real dough loaf that undergoes baking. There is no formation of an external crust and an 181 internal true crumb. One therefore may reasonably raise the question: Can a single DSC investigation shed some light 182 on the changes that take place in a real baking process? The obvious the answer is "no". 183

188 To overcome the substantial differences between a DSC experiment and a real baking test, one has to perfect the 189 information through other investigations, like DSC and TG runs performed with open cells [25], including Knudsen 190 cells [26], and rheological tests [27]. However, in such 191

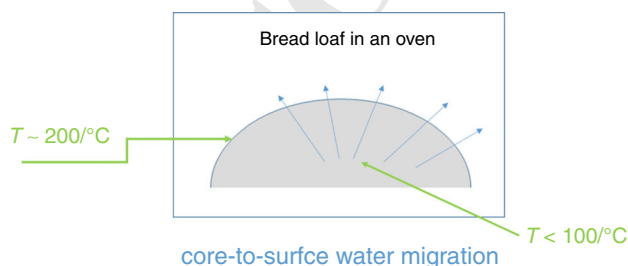


Fig. 1 Water displacement within a bread loaf undergoing baking in an oven

193 conditions the large exothermal effect related to the water 194 evaporation overwhelms any other heat exchange just in 195 the temperature range where most of the interesting 196 transformations take place. One cannot therefore rely on 197 the overall thermal effect, but shall first take into account 198 mass loss and temperatures of the main signals. Knudsen 199 isothermal TG allows the estimation of the relative 200 humidity (RH) of starting and final products [28]. Such 201 multifacet approach allows one to recognize that starch 202 gelatinization is not complete in most regions of the bread 203 loaf, above all the crust, where the quick drying makes the 204 water content insufficient to sustain the process [26]. This 205 picture comes from DSC and TG investigations that allow 206 definition of a TTT (time, temperature, transformation) 207 diagram related to the starch gelatinization in a dough that 208 undergoes baking [25].

209 The TTT diagram (Fig. 2) can be determined with 210 several DSC investigations, each at a given heating rate. 211 From every single DSC run, one can draw the corresponding 212 trend of the gelatinization degree, $\alpha[T(t)]$, sweeping the area 213 beneath the DSC signal [25]. One has, however, to take into 214 account the simultaneous loss of moisture, which can be 215 assessed with parallel TG investigations [25]. 216

217 The RH is about 98% in the starting dough, but it drops 218 below 80% in the crust and levels at about 95% in the 219 crumb [28]. The gluten meshwork traps almost 15% of the 220 moisture content of the crumb, while the rest of the moisture 221 content, shared by starch and non-starch polysaccharides 222 and globular proteins, undergoes easier displacements [29]. 223 According to TG investigations, a starchy gel releases its 224 moisture content continuously, producing a single DTG peak, 225 while a gluten/water aggregate shows a double peak [29], 226 which indicates the

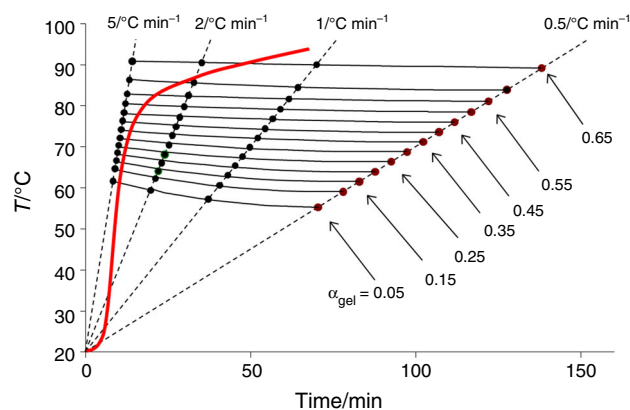


Fig. 2 TTT diagram related to the progress of starch gelatinization, $\alpha = \alpha(T)$, within a bread loaf undergoing baking. The red line reflects the actual thermal history at the loaf core. The straight dotted lines correspond to the DSC traces recorded at different heating rates (0.5, 1, 2 and 5 °C min⁻¹) while full tie lines are drawn across iso- α steps. Data from ref [25]

227 presence of at least two moisture fractions released below
 228 and above 100 °C, respectively. The occurrence of a high-
 229 temperature peak indeed is the fingerprint of the presence
 230 of gluten (Fig. 3) and can be an easy check of many
 231 “gluten-free” products [20]. However, the moisture frac-
 232 tion bound to gluten decreases when the dough is over-
 233 kneaded, namely when the gluten meshwork undergoes a
 234 mechanical stress, but comes back to the starting level after
 235 a two-hour rest [29]. If the overstressed dough immediately
 236 undergoes baking, it releases a larger amount of water and
 237 the final product is drier and crispier (e.g., biscuit) [30].
 238 The underlying reason is that weak driving forces, like
 239 those experienced in kneading and extruding, can displace
 240 a large fraction of water linked to polymers, so allowing an
 241 easier realignment of polymer chains and the formation of
 242 more tight supramolecular clusters or networks.

243 Since the vapor phase is pure water, the equilibrium
 244 condition with a condensed aqueous phase implies the
 245 equivalence of the water chemical potential, μ_W :

$$\mu_{W,vap}^* = \mu_{W,liq}^* + RT_{vap} \ln(a_W)$$

247 that is

$$T_{vap} = \frac{\Delta_{vap} \mu^*(T)}{R \ln(a_W)}$$

249 where “*” and R stand for “pure water” and the gas
 250 constant, respectively. Reminding that $a_W \leq 1$ and Δ_{vap} -
 251 $\mu^*(T) < 0$ for $T > T_{vap}^*$ (i.e., 373.15 K), a lower a_W
 252 implies a higher T_{vap} , as $\ln(a_W)$ has a steeply decreasing
 253 trend. When the temperature of the system is not far from
 254 the relevant T_g , a real equilibrium is never reached. This
 255 means that the system can host regions with different water
 256 activity and different temperature of vaporization, T_{vap} .
 257 This conclusion is in line with the results of NMR relax-
 258 ation experiments [9].

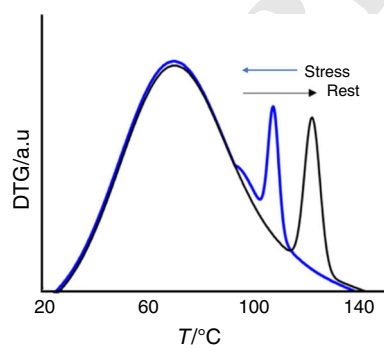


Fig. 3 DTG trace from a wheat flour dough. The high T peak concerns the moisture fraction bound to gluten. The temperature of this peak decreases when the dough undergoes a mechanical stress, like over kneading, but turns back to the original value after two-hour rest at room temperature

259 The crumb sealed in a plastic bag does not lose mois-
 260 ture, but undergoes staling that implies short-range dis-
 261 placements of water because of the formation of
 262 amylopectin and amylose crystal phases [31]. Similar
 263 changes occur in pasta and other starchy products [32].

264 As a result, water activity decreases in a staling crumb
 265 [28] even when any water loss is prevented and the product
 266 hardens since the relevant glass transition rises above room
 267 temperature because mostly the plasticizing water migrates
 268 into the polymer crystal zones. A 24-h stale crumb can
 269 release the moisture fraction fixed by gluten only when
 270 heated up to 175 °C (Fig. 4), while practically all the
 271 moisture bound to the other polymers leaves the crumb at
 272 temperatures below 100 °C [28].

273 Minor additions of extra ingredients produce substantial
 274 changes. For example, the addition of non-starch carbo-
 275 hydrates, like arabino-xylans, to the original wheat dough
 276 implies reduction of the gluten-bound moisture fraction
 277 and a final crumb with coarser alveolar structure and
 278 slower staling [24]. The addition of gluten-free flours (like
 279 soy, buckwheat, rice) delays the denaturation threshold of
 280 the globular proteins, whose exothermic effect can be
 281 “disentangled” from the endothermic one related to the
 282 starch gelatinization in DSC traces [20], and produces a
 283 denser loaf with smaller alveoli (although in a larger
 284 number per mL).

285 These pieces of information coupled with extra data,
 286 like elastic and rheological moduli [18], support some
 287 reliable conclusions about the macroscopic changes
 288 occurred in the system. NMR and X-ray diffraction provide
 289 evidence of changes at the molecular level either in the
 290 crust or in the crumb regions of the loaf [7, 9, 33–36].

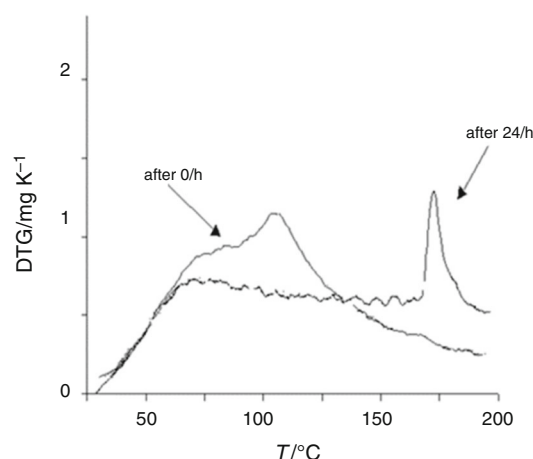


Fig. 4 DTG traces of stale crumb sealed in a plastic bag for 0 and 24h. Data from ref [28]

291 **Starch and other components**

292 Much simpler is the study of the behavior of single
293 ingredients of the dough, like an aqueous suspension of
294 starch granules, requiring just standard DSC investigations
295 to monitor the progress of the starch gelatinization in dif-
296 ferent conditions, namely water content, salt concentration,
297 presence of proteins, or fats, etc. MTDSC [37] provides a
298 better evidence especially when the investigation implies
299 heating/cooling cycles (Fig. 5).

300 These studies show that the starting heterogeneity of the
301 system (aqueous suspension of starch granules) becomes a
302 mixed amylose/amylopectin gel that, when heated above
303 85 °C, becomes a sol system containing some amylose
304 crystals and, in the presence of endogenous fats, amylose-
305 lipid complexes. These complexes undergo fusion at about
306 110 °C, while the amylose crystals melt above 135 °C
307 [38]. On cooling from 150 °C to room temperature, the
308 amylose/lipid complexes undergo a partial restoration, and
309 the final system is an amylopectin gel containing three
310 types of crystals (amylose crystals, amylose/lipid

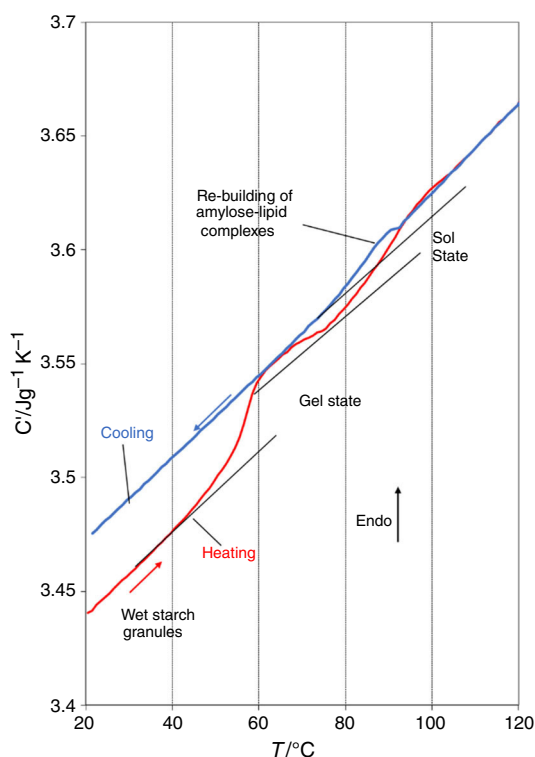


Fig. 5 Heating-cooling MTDSC traces of the in-phase heat capacity, C' , of an aqueous suspension of wheat starch granules. The red trace is the first heating run, while the blue one corresponds to the cooling run. The evidence clearly shows the irreversibility of the starch gelatinization, while starch-lipid complexes (lipids are always associated with wheat starch granules) undergo fusion on heating and at least a partial reconstruction on cooling. Data from Ref. [38]

complexes, amylopectin crystals) that show different X-ray
diffraction patterns [7].

Specific investigations devoted to check the role or the
effect of ionic strength, or simple sugars, on starch gela-
tinization, showing that cations and anions produce dif-
ferent effects [39] on starch gelatinization, while the
addition of simple sugars normally delays the onset of the
gelatinization and reduces its extent [40]. These results are
relevant to nutritional issues, especially those related to
diabetic consumers.

Non-aqueous food systems: the case of cocoa butter

Fats are the major family of non-aqueous food components
(others are terpenes, hydrophobic vitamins, some aroma
compounds, etc.). Many real food products can contain
separate aqueous and fat layers or finely disperse phases
(emulsions), or mechanically mixed ice and lipid crystals
(ice cream), or mixtures of different lipids (edible oil,
butter, margarine, etc.). The study of such systems once
again requires the disentanglement of different processes
that can occur simultaneously. The standard approach
obviously is the investigation of single substances or
mixtures of homologous compounds. An interesting system
to study is cocoa butter, which indeed is a mixture of tri-
acylglycerols (TAG) that behaves like a solid solution with
its own polymorphic crystal phases. These show a mono-
tropic behavior, namely each crystal phase has its own
melting point and there are no transition temperatures
between polymorphs. According to Wille and Lutten [41],
there are six polymorphs with different melting points and
fusion enthalpies (Table 1).

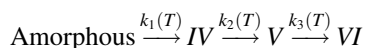
The DSC evidence is an endothermic signal that corre-
sponds to the fusion of the crystals. In the case of cocoa-
based products, a large number of DSC traces, collected to
investigate the TAG polymorphous transitions in cocoa
butter, cocoa liquor and dark chocolate, showed that these
transitions have an extent and occur with a kinetics that
largely depend on the previous thermal history [42]. The

Table 1 TAG polymorphs in cocoa butter according to [41]

Polymorph	$T_{fus}/^{\circ}\text{C}$	$\Delta_{fus} H/\text{Jg}^{-1}$
I	17.3	Not appl.
II	23.5 ± 1.0	86.15
III	26.0 ± 0.5	112.47
IV	29.0 ± 0.5	117.47
V	31.3 ± 0.5	136.73
VI	36.0 ± 1.5	148.02

349 contribution of the various polymorphs to the DSC endo-
350 therm of cocoa butter samples that had undergone a pre-
351 vious thermal history, namely annealing at various
352 temperatures, comes out through a deconvolution treatment
353 (Fig. 6).

354 The results are at the **fundamental** of a kinetic model
355 [42] that describes the progress of the TAG evolution
356 toward the most stable crystal form,



358 The kinetic model predicts the final crystal phases after
359 any annealing history [42]: a result of obvious interest to
360 explain the blooming of chocolate during the shelf life [43]
361 and plan the industrial production process.

362 As mentioned, complementary information collected
363 through other experimental approach can perfect the
364 description of the system. Laser confocal microscopy and
365 rheology investigations [44] demonstrated that TAG
366 polymorphism reflects a hierarchical scale of structures at
367 the mesoscopic level: Aggregates of small crystals form
368 domains (spherulites) linked to one another in a fractal
369 network that hosts a liquid (amorphous) phase within its
370 meshes. The fractal dimension of the network would
371 depend on the size of the spherulites, which in turn depends
372 on the growth extent of the specific TAG polymorph(s),
373 determined by the cooling rates and annealing tempera-
374 tures. The final mesoscopic structure of the system (size of
375 spherulites, fractal dimensions and fraction of amorphous/
376 liquid phase) affects the rheological properties of the
377 products.

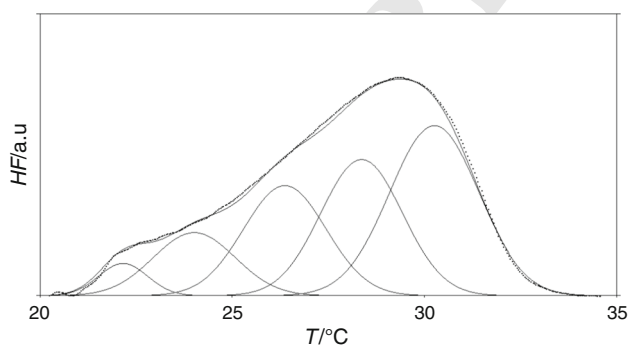


Fig. 6 Deconvolution of the endothermic DSC signal of the fusion of a cocoa butter sample. The Gaussian peaks correspond to the contributions of different TAG polymorphs (I, II, III, IV and V) present in the sample after a given annealing treatment. Not published data (experimental details in Ref. [42])

Shelf life and microbial spoilage

378

379 Another field of application of TAC in food science con-
380 cerns the shelf life, during which food products undergo
381 many changes of structure, texture, composition, etc.,
382 related to a number of factors, like formation of crystal
383 phases, dehydration, oxidation, microbial spoilage.

Structural and composition changes

384

385 Food products undergo physical changes, as they are
386 metastable systems tending to attain more or less slowly
387 some equilibrium state. This is typical of dispersed systems
388 [45]. Foams, emulsions and suspensions tend to collapse
389 and separate in bulk phases. Amorphous solids tend to
390 crystallize whenever the local molecular mobility is large
391 enough to allow short-range displacements. Water migrates
392 along local gradients of chemical potential across phase
393 boundaries and/or escape toward the head-space of the
394 system. These changes produce substantial modifications
395 of texture, color, taste and flavor and often make the pro-
396 duct unacceptable by the consumer. A good example
397 comes again from starchy products that undergo staling
398 [16, 18]. TAC experiments allow a quantitative assessment
399 of this change and its progress on ageing (see above).

400 The most important composition change that affects the
401 properties of food is the decrease in the moisture content.
402 The change is often desired as drying is an old practice to
403 preserve food.

404 Besides thermal and freeze-dry dehydration, osmo-de-
405 hydration is a mild treatment that does not severely affect
406 the original properties of the starting product, as in the case
407 of fruits and vegetables whose moisture content can
408 account for more than 85% of the overall mass. The pro-
409 cess requires the use of a hypertonic medium where to pour
410 the food. The consequent displacement of water affects
411 both the extra- and the intracellular regions. Actually, a
412 gradient of water activity between the hypertonic medium
413 and the extracellular region drives water toward the former
414 (whose composition remains practically unaffected because
415 of its overwhelming mass). Since there is no semiperme-
416 able barrier between these fluids, some solute (e.g., sugar)
417 of the hypertonic medium enters the extracellular region
418 and remains there at the end of the process. The intracel-
419 lular water trespasses the cell membranes toward the
420 extracellular region. Because of this moisture depletion, the
421 fruit shrinks keeping its basic structure, which can turn
422 back to the starting status almost completely on rehydra-
423 tion, save for the trapped sugar. Knudsen TG is a suit-
424 able tool to monitor the process and provides data that
425 allow description of all its steps, including the shrinkage
426 [14]. The corresponding DTG trace reflects the flux, J_w , of

427 escaping water that depends on the gradient of the chemical
428 potential, $\nabla\mu_w$,

$$J_w = \frac{1}{A} \frac{dm}{dt} = \frac{DTG}{A} \propto -D_w \nabla\mu_w$$

430 where A and D_w stand for cross-sectional area and diffu-
431 sion coefficient, respectively. The dehydration of a sample
432 in Knudsen regime implies the simultaneous detection of
433 water activity and water content, which therefore allows
434 the direct assessment of the dehydration isotherm [26]. If
435 one performs the Knudsen dehydration of partially osmo-
436 dehydrated samples, he can accordingly define both the dry
437 mass and the water activity at every intermediate step of
438 the process. It comes out that the desorption trend of the
439 osmo-dehydrated food tends to overlap that of the hyper-
440 tonic medium (Fig. 7) for large drying extents [14].

441 This reflects the decrease in the overall mass of the
442 intracellular region. Since the three fluids (hypertonic
443 medium, extra- and intracellular regions) must have the
444 same water activity at any step of the process, the gap
445 between the desorption trends of non-treated sample and
446 hypertonic medium can be used to predict the mass pro-
447 portion between intra- and extracellular regions in a par-
448 tially osmo-dehydrated sample applying the classic lever
449 rule [14]. This finally leads to a likely estimation of the
450 shrinkage of the sample, assuming that the volume of the
451 moisture lost is the main contribution [14] to the process.

452 Oxidation

453 Another crucial issue related to the shelf life of most food
454 products is oxidation. The addition of antioxidants, vac-
455 uum-sealing or inert atmosphere can delay the process.
456 Some food products are naturally rich of antioxidant

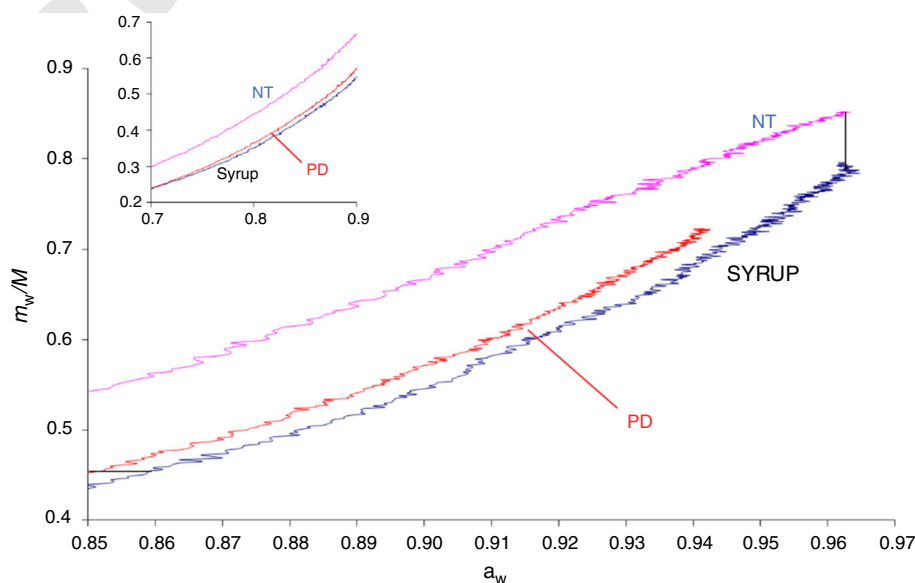
457 compounds and therefore are easier to preserve. However,
458 because of the multi-component and multi-phase nature of
459 most food, a quantitative assessment of the antioxidant
460 potential of a given product is not easily achievable. In
461 most cases, such evaluation comes from experimental data
462 drawn with independent methods, most of which require
463 preliminary time-consuming treatments.

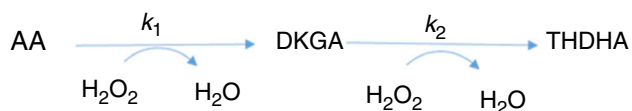
464 Reaction calorimetry (RC) allows determination of the
465 exothermic effect related to the scavenging of oxygen or
466 oxidants, regardless of the physical form of the sample and
467 without the need of sample pretreatments. RC can therefore
468 be of help to describe the behavior of the antioxidant
469 potential of many food systems [46].

470 A common natural antioxidant is ascorbic acid (AA).
471 When matched with H_2O_2 , AA (one should better say
472 ascorbate anion) produces a large exothermic effect that,
473 for small AA concentrations ($[AA] \leq 500$ mM), appears
474 as a single peak in the isothermal RC trace. Nonetheless, at
475 larger concentrations, the signal shows a shoulder that
476 suggests an underlying multi-step reaction mechanism. It is
477 known that the oxidation of AA occurs via radical inter-
478 mediates with the formation of dehydroascorbic Acid
479 (DHA), diketogulonic acid (DKGA) and the end product
480 4,5,5,6-tetra hydroxyl-2,3-di-keto-hexanoic acid
481 (THDHA). Each step would imply its own thermal effect
482 that contributes to the overall signal detected. For the sake
483 of simplicity, a simpler scheme of two consecutive steps
484 involving the known intermediate compounds may be of
485 help to suggest an interpretation of the signal (Scheme 2).

486 This scheme implies a simple solution and a corre-
487 spondent expression for the related heat flow:

Fig. 7 Knudsen desorption isotherms of non-treated (NT) and partially osmo-dehydrated apple pulp (PD), compared with that of the hypertonic syrup used for the osmo-dehydration. The inset shows that the desorption trend of PD tends to overlap the one of SYRUP for large dehydration extents. Adapted from Ref. [14]





Scheme 2 Simplified reaction steps of the oxidation of AA with hydrogen peroxide [46]

$$\dot{Q} = \left[k_1 \Delta H_1 + \frac{k_1 k_2}{k_2 - k_1} \Delta H_2 \right] e^{-k_1 t} - \left[\frac{k_1 k_2}{k_2 - k_1} \Delta H_2 \right] e^{-k_2 t}$$

$$= a e^{-c t} - b e^{-d t}$$

489 The best fit of the RC trace leads to recognize that
490 $\Delta H_1 < 0$ and $\Delta H_2 > 0$. Accordingly, one can split the
491 overall signal in two contributions,

$$k_1 \Delta H_1 e^{-k_1 t} = dQ_1/dt$$

$$\frac{k_1 k_2}{k_2 - k_1} \Delta H_2 [e^{-k_1 t} - e^{-k_2 t}] = dQ_2/dt.$$

493 Figure 8 reports an example of such split.

494 Having so defined the oxidation process of AA, one can
495 determine the antioxidant power of a given food product
496 (usually wine, fruit juices or liquid products) versus
497 hydrogen peroxide matching the heat released with that
498 obtained with known amounts of ascorbic acid (AA) at
499 given pH and temperature conditions. This procedure
500 allows the assessment of a rank of antioxidant capacity of
501 various food products, expressed as ascorbic acid equivalents [46]. Similar investigations concern spent coffee [47],
502 tocopherols [48] and linoleic acid [49].
503

504 Microbial spoilage

505 The most important changes during the shelf life depend on
506 the microbial spoilage, which takes place when the relative
507 humidity (RH) is larger than 70% (lower thresholds apply

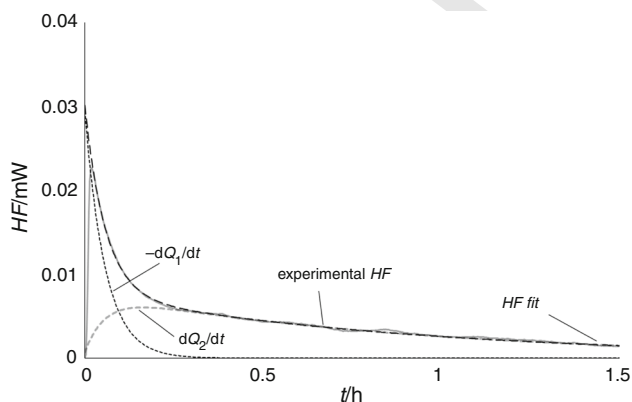


Fig. 8 Split of the RC isothermal trace of AA oxidation with excess H_2O_2 in the sum of an exo- and an endothermic contribution. $[\text{AA}] = 0.25 \text{ mM}$. Not published data (experimental details in Ref. [46])

for molds and enzymatic processes) [50, 51]. This is why
the control of RH is compulsory for food technologists.

Isothermal calorimetry (IC) is an ideal tool to monitor
the presence and the growth of microbial organisms, no
matter whether single or many microbial strains are present,
in almost any kind of medium and represents a valid
alternative to the plate counts, not requiring any preliminary
treatment [52].

The increase in the microbial population is the neat
balance between growth (exothermic) and death (normally
endothermic) of cells, although the former implies a much
larger thermal effect. Beside the neat exothermic balance
related to the growth progress, one should account for the
fact that, in a microbial culture, some aged cells do not
duplicate, but still are able to uptake and metabolize the
available substrate, which implies an extra neat exothermic
effect proportional to the number of viable cells [53].
Therefore, the observed thermal effect, \dot{Q} , appears as a
large exothermic signal that reflects the growth progress
and the non-growth cell metabolism, namely

$$\dot{Q} = \dot{N}q_g + N\dot{q}_m$$

where q_g and \dot{q}_m are the heat released by a single duplicating cell and the metabolic non-growth heat flow of a single viable cell, respectively, while N and \dot{N} stand for number of viable cells and growth rate. However, the calorimetric signal appears only when \dot{Q} is significantly larger than the detectability threshold of the instrument used, namely $0.1 \mu\text{W/mL}$ for standard calorimeters [54]. This implies that the onset of the calorimetric signal occurs substantially later than the onset of the growth trend and corresponds to the attainment of the detectability threshold of the instrument (Fig. 9).

During the span between growth and signal onsets, both the microbial population within the calorimetric cell and

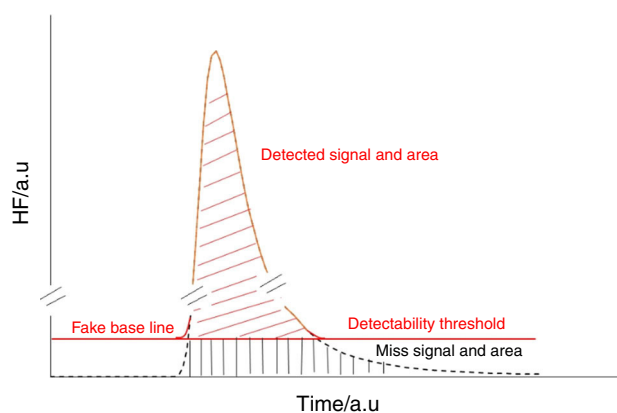


Fig. 9 The onset of the calorimetric trace can appear rather smooth and broad: this shape has nothing to do with the actual trend of the growth, but simply reflects the trespass through the detectability threshold

542 the growth rate increase by some orders of magnitude.
 543 When the microbial population approaches its end steady
 544 level, the growth rate vanishes. The relevant thermal effect
 545 can again drop below the detectability threshold, unless the
 546 metabolic contribution $N\dot{q}_m$ is sufficiently large because of
 547 the high value attained by N . In other words, IC only
 548 partially “perceives” the growth progress at its beginning
 549 and at its end. The so-called lag phase that usually precedes
 550 the growth onset is expected to imply a very low heat flux
 551 (less than 0.1 fW/CFU), therefore remaining hardly
 552 detectable even with very sensitive instruments.

553 A critical issue to consider is that microbial growth in
 554 food systems is not the same as in planktonic conditions,
 555 *i.e.*, cells dispersed in an aqueous solution of nutrients. In
 556 real systems, the progress of the microbial growth can
 557 substantially modify the surrounding environment imply-
 558 ing change of pH, ionic strength, substrate concentration
 559 [55] and, of course, the available volume to accommodate
 560 the new cell generations.

561 To achieve a reliable view of what is happening in a real
 562 system, one needs to perform separate experiments with a
 563 single microbial species in planktonic conditions and check
 564 the changes produced by varying the medium pH, the
 565 temperature and, tentatively, by adding some extra
 566 microbial species or adverse compounds [56]. Of great help
 567 are extra data, possibly collected from the same system that
 568 is undergoing the calorimetric investigation (Fig. 10). This
 569 implies the use of special calorimetric cells that allow the
 570 insertion of extra sensors to detect pH, color changes, or
 571 the concentration of given probe compound [55].

572 Finally, one has to translate all these pieces of infor-
 573 mation in a clear picture of the microbial growth that may
 574 occur in a real system. For example, one may attempt an
 575 interpretation of the experimental overall evidence through
 576 models of microbial growth [57, 58]. However, the

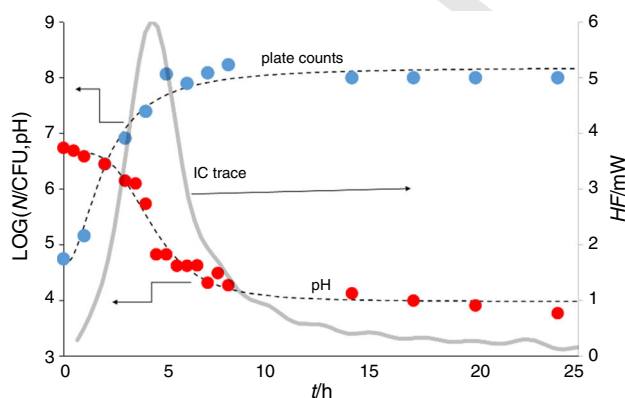


Fig. 10 *Streptococcus thermophilus*. IC trace, plate counts and pH Adapted from ref [55]: fitting dotted lines for plate counts and pH according to the semiempirical model (see text below) and dose-logistic model, respectively

577 assessment of N and \dot{N} is unachievable through an “a
 578 priori” approach, as the number of variables involved in
 579 real systems is large and can differ from case to case
 580 [59, 60]. An interpretation of the calorimetric signal (and
 581 experimental evidences of the traditional microbiology
 582 investigations) that does not require the use of a growth
 583 model is therefore of great interest.

584 A semiempirical approach, based on some experimental
 585 evidence, like plate counts, has proven rather adequate
 586 whenever the microbial strain grows via duplication
 587 mechanism [61, 62], namely $N = N_0 2^{t/\tau}$, where N_0 and τ
 588 (t) are the starting microbial population and the generation
 589 time, respectively (Fig. 11).

590 It is important to notice that the plate count data are
 591 commonly reported in a semilogarithmic scale, the slope of
 592 which corresponds to the so-called specific growth rate,
 593 \dot{N}/N , while the calorimetric signal is related to the absolute
 594 growth rate, \dot{N} , which shows a maximum quite later than
 595 the former, and to N .

596 In the considered example, N_0 is close to 10^2 CFU,
 597 while the end steady values of N are about 10^9 CFU. This
 598 means that the end tail of the IC trace would reflect the
 599 microbial non-growth metabolism if \dot{q}_m is larger than 0.1
 600 fW/CFU.

601 The proposed semiempirical model suggests the fol-
 602 lowing fitting expression for the IC trace:

$$\dot{Q} = q_g \left[N_0 \times 2^{t/\tau} \times \log_e(2) \times \frac{\tau - t\tau}{\tau^2} \right] + \dot{q}_m \left[N_0 \times 2^{t/\tau} \right]$$

604 where $\tau = \left(\frac{a}{t} + bt\right)$ is the generation time [61, 62]. To
 605 account for the delayed onset of the IC signal, one has to
 606 replace t with $(t - \Delta t)$ in every expression used. This is
 607 tantamount as to replace the microbial culture with a vir-
 608 tual one with the same starting N_0 , and a generation time

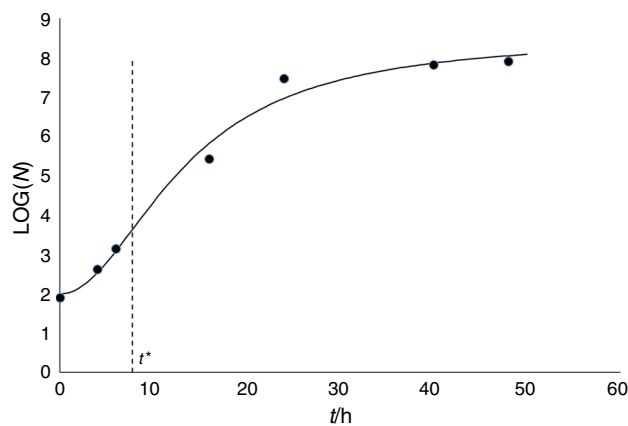
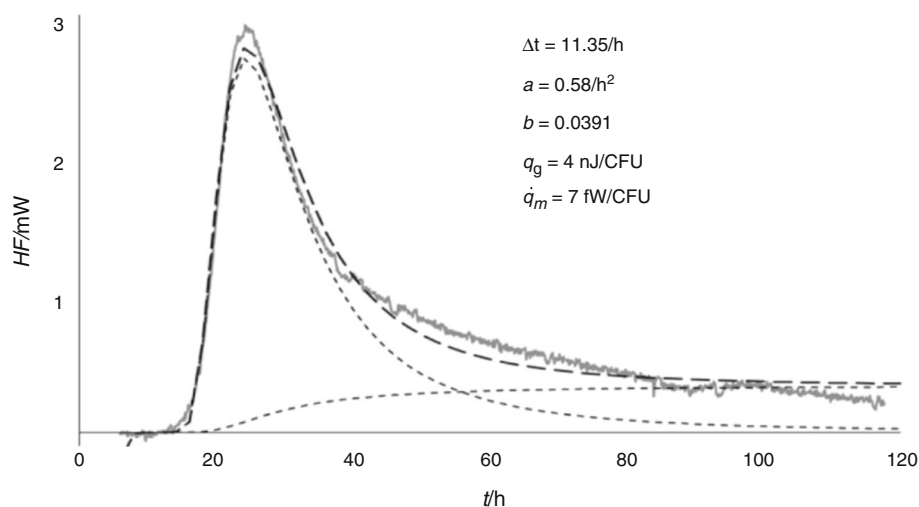


Fig. 11 An application of the proposed empirical model to fit the experimental plate counts of *L. helveticus*. The maximum of the specific growth rate (\dot{N}/N) occurs at $t = t^*$, when the growth progress reaches $1/4$ of the overall growth span in logarithmic scale [61]

Fig. 12 IC trace of a culture of *L. helveticus* (10^3 CFU in 6 mL, at 37 °C). The heavy dashed line corresponds to the fit obtained with the proposed semiempirical model (see text) that allows the splitting of the signal in growth and non-growth contributions (dotted lines). The inset reports the best-fitting parameters



609 that is a little larger in the early phase of the growth (that
610 anyway escaped from the IC detection) but becomes a little
611 smaller during the so-called exponential growth phase. The
612 parameters Δt , a , b , q_g and \dot{q}_m come from the fitting rou-
613 tine. Figure 12 shows the result.

614 The reliability of the q_g and \dot{q}_m values is obviously
615 limited to the respective order of magnitude as they come
616 from the product between very large (N or \dot{N}) and very
617 small (q_g or \dot{q}_m) quantities. Nonetheless, they can be of
618 some interest for those who are involved in the study of cell
619 biochemistry. These values of q_g and \dot{q}_m allow estimation
620 of the threshold IC detectable levels of N and \dot{N} , namely
621 10^6 CFU/mL and 5 CFU $\text{mL}^{-1} \text{s}^{-1}$, respectively.

622 A threshold value of 10^6 CFU/mL may seem rather high,
623 casting some doubts about the practical use of calorimetry
624 to monitor microbial growth. However, it is not so when
625 considered in the appropriate perspective: Namely, real
626 situations imply population densities of this (or higher)
627 order of magnitude that are too large for the standard
628 practice of the microbiological plate counts. Plate counts
629 indeed require a previous dilution of the sample, which
630 implies loss of accuracy. The calorimetric approach does
631 not require a previous dilution of the original sample that
632 can directly undergo the calorimetric investigation without
633 any preliminary preparation.

634 Conclusions

635 Some examples of TAC investigations applied to food
636 products or their ingredients show that these experimental
637 approaches, combined with side information from other
638 techniques, allow one to shed light on the properties of
639 these multi-phase and multi-component systems. However,
640 the investigator needs to put at work a specific expertise to

select the suitable instrument and envisage an adequate
experimental plan, according to the available tools.

A special attention requires the interpretation of the
collected data, as food systems undergo simultaneous and
concurrent changes that produce an overall instrumental
output, which may appear neat and simple, but actually is
the resultant of many contributions.

The need of separate investigations dealing with simple
model systems is a way to disentangle different transfor-
mations that occur in the same temperature range or time
span. Mathematical deconvolution of the recorded signals
is a major tool to use, but the underlying physical models
require a critical scrutiny to avoid oversimplifications or
imply unsuitable assumptions.

Kinetic models can usually be of help, because of the
intrinsically dynamic nature of the system under study, as
in the case of microbial cultures. However, semiempirical
approaches seem more reliable than a priori schemes,
since, although not providing general “laws”, they can
account for the peculiarities of the system under study
through adjustable fitting parameters, whose physical
meaning may appear a posteriori [61, 62].

References

1. Schiraldi A, Lilley TH, Braibanti A, Ollivon M, Cesaro A, Masi P. Calorimetry, thermal analysis and chemical thermodynamics in food science: Report on the panel discussion. *Thermochim Acta*. 1990;162:253–64. 665
2. Applications of calorimetry and thermal-analysis to food systems and processes. *Thermochim Acta*, 246 (1994) Special Issue, R11-R12, guest Ed. A. Schiraldi. 666
3. Schiraldi A, Piazza L, Fessas D, Riva M, in Handbook of thermal analysis and calorimetry (1999) chap. 16, R. Kemp Ed., Elsevier Publ., Amsterdam, 829–921. 667

- 675 4. Slade L, Levine H. Beyond water activity: recent advances based
676 on an alternative approach to the assessment of food quality and
677 safety. *Critical Rev Food Sci Nutr*. 1991;30:115–360.
- 678 5. Tolstoguzov VB. Some thermodynamic considerations in food
679 formulation. *Food Hydrocolloids*. 2003;17:1–23.
- 680 6. Bubbles in food, (1999), G.M. Campbell, C. Webb, S.S. Pandiella
681 and K. Nirajan, Eds., Eagan Press Publ.
- 682 7. Schiraldi A, Fessas D, Signorelli M. Water activity in biological
683 systems—a review. *Pol J Food Nutr Sci*. 2012;62:5–13.
- 684 8. Zobel HF. Starch crystal transformations and their industrial
685 importance. *Starch*. 1988;40:1–7.
- 686 9. Hills BP Water management in the design and distribution of
687 quality foods”, (1999) Y.H. Roos, R.B. Leslie and P.J. Lillford
688 Eds., Technomic Publ. Co., Lancaster, Penn., USA, 107–131.
- 689 10. Beltonen PS. *J Cereal Sci*. 1999;29:103–7.
- 690 11. Schiraldi A, Piazza L, Riva M. Bread staling: a calorimetric
691 approach. *Cereal Chem*. 1996;73:32–9.
- 692 12. Schiraldi A, Fessas D, Signorelli M, in “Calorimetry in food
693 processing”, G. Kalentuc Ed., IFT Press series (2009), chap 11.
- 694 13. Krokida MK, Karathanos VT, Maroulis ZB. Effect of freeze-
695 drying conditions on shrinkage and porosity of dehydrated agricul-
696 tural products. *J Food Eng*. 1998;35:369–80.
- 697 14. Lewicki PP. Design of hot air drying for better foods. *Trends*
698 *Food Sci Technol*. 2006;17:153–63.
- 699 15. Pani P, Schiraldi A, Signorelli M, Fessas D. Thermodynamic
700 approach to osmo-dehydration. *Food Biophys*. 2010;5:177–85.
- 701 16. Roos HY. Water activity and physical state effects on amorphous
702 food stability. *J Food Process Preserv*. 1993;16:433–47.
- 703 17. Roos HY. Phase transitions in foods. San Diego: Acad. Press Inc.;
704 1995.
- 705 18. H. This, “Molecular Gastronomy: exploring the science of flavors”
706 (2005), ISBN: 023114170X.
- 707 19. Larsson H, Eliasson A-C. *Cereal Chem*. 1996;73:18–31.
- 708 20. Fessas D, Signorelli M, Pagani A, Mariotti M, Iametti S, Schir-
709 aldi A. Guidelines for buckwheat enriched bread: thermal anal-
710 ysis approach. *J Therm Anal Cal*. 2008;91:9–16.
- 711 21. Tolstoguzov VB. Foods as dispersed systems. Thermodynamic
712 aspects of composition-property relationships in formulated food.
713 *J Therm Anal Cal*. 2000;61:397–409.
- 714 22. Piazza L, Masi P. Moisture redistribution throughout the bread
715 loaf during staling and its effects on mechanical properties.
716 *Cereal Chem*. 1995;72:320–5.
- 717 23. Mitchell JR, Fan JT, Blanshard JMV. Bubbles in food (1999),
718 G.M. Campbell, C. Webb, S.S. Pandiella and K. Nirajan, Eds.,
719 Eagan Press Publ.
- 720 24. Fessas D, Schiraldi A. Texture and staling of wheat bread crumb:
721 effects of water extractable proteins and pentosans’. *Thermochim*
722 *Acta*. 1998;323:17–26.
- 723 25. Fessas D, Schiraldi A. Starch Gelatinization Kinetics in Bread
724 Dough. DSC investigations on simulated baking processes.
725 *J Therm Anal Cal*. 2000;61:411–23.
- 726 26. Schiraldi A, Fessas D. Classical and Knudsen thermogravimetry
727 to check states and displacements of water in food systems.
728 *J Therm Anal Cal*. 2003;71:221–31.
- 729 27. Schiraldi A, Piazza L, Brenna O, Vittadini E. Structure and
730 properties of bread dough and crumb. *J Therm Anal*.
731 1996;47:1339–60.
- 732 28. Fessas D, Schiraldi A. Water properties in wheat flour dough II:
733 classical and Knudsen thermogravimetry approach. *Food Chem*.
734 2005;90:61–8.
- 735 29. Fessas D, Schiraldi A. Water properties in wheat flour dough I:
736 classical thermogravimetry approach. *Food Chem*.
737 2001;72:237–44.
- 738 30. Piazza L, Schiraldi A. Correlation between fracture of semi-sweet
739 hard biscuits and dough viscoelastic properties. *J Texture Stud*.
740 1997;28:523–41.
- 741 31. Schiraldi A, Fessas D. Bread staling (2000), P. Chinachoti, Y.
742 Vodovotz, Eds., CRC, Boca Raton, FL, 1–17.
- 743 32. Riva M, Fessas D, Schiraldi A. Starch retrogradation in cooked
744 pasta and rice. *Cereal Chem*. 2000;77:433–8.
- 745 33. Kou Y, Ross EW, Taub LA. Amorphous food and pharmaceutical
746 systems, (2002) H. Levine Ed., The Royal Society of Chemistry,
747 Cambridge, 48–58.
- 748 34. Hall L-D, Amin MHH, Evans S, Nott KP, Sun L. Water science
749 for food, health, agriculture and environment, Z. Berk, R.B.
750 Leslie, P.J. Lillford and S. Mizrahi Eds., Technomic Publ.,
751 Lancaster, Penn., USA, 255–271.
- 752 35. Schiraldi A. Starch and starch containing products: origins -
753 structure, properties and new technologies”, V. Yuryev, A.
754 Cesaro and W. Bergthaller Eds., Nova Science Publishers, (2002)
755 chap 20, 287–296.
- 756 36. Vodovotz Y, Vittadini E, Sachleben JR. Use of 1H cross-relax-
757 ation nuclear magnetic resonance spectroscopy to probe the
758 changes in bread and its components during aging. *Carbohydr*
759 *Res*. 2002;337:147–53.
- 760 37. Schiraldi A, Fessas D, Signorelli M, data presented at ESTAC 9,
761 Krakow, August 27–31, 2006.
- 762 38. Yuryev VP, Krivandin AV, Kiseleva VI, Wasserman LA,
763 Genkina NK, Fornal J, Błaszczak W, Schiraldi A. Structural
764 parameters of amylopectin clusters and semi-crystalline growth
765 rings in wheat starches with different amylose content. *Carbohydr*
766 *Res*. 2004;339:2683–91.
- 767 39. Lii CY, Lee BL. *Cereal Chem*. 1993;70:188–92.
- 768 40. Hedayati S, Shahidi F, Koocheki A, Farahnaky A, Majzoobi M.
769 Physical properties of pregelatinized and granular cold water
770 swelling maize starches at different pH values. *Int J Biol Mol*.
771 2016;88:499–504.
- 772 41. Wille RL, Lutton ES. Polymorphism of cocoa butter. *J Am Oil*
773 *Chem Soc*. 1966;43:491–6.
- 774 42. Fessas D, Signorelli M, Schiraldi A. Polymorphous transitions in
775 cocoa butter: a quantitative DSC study. *J Therm Anal Cal*.
776 2005;82:691–702.
- 777 43. Aguilera JM, Michel M, Mayor G. Fat migration in chocolate:
778 diffusion or capillary flow in a particulate solid?—a hypothesis
779 paper. *J Food Sci*. 2004;69:167–74.
- 780 44. Narine SS, Marangoni AG. Relating structure of fat crystal net-
781 works to mechanical properties: a review. *Food Res Int*.
782 1999;32:227.
- 783 45. Tolstoguzov VB. Texturising by phase separation. *Biotechnol*
784 *Adv*. 2006;24:626–8.
- 785 46. Kamrul HSM, Schiraldi A, Cosio MS, Scampicchio M. Food and
786 ascorbic scavengers of hydrogen peroxide. *J Therm Anal Cal*.
787 2016;125:729–37.
- 788 47. Haman N, Ferrentino G, Imperiale S, Scampicchio M. Antioxi-
789 dant and prooxidant activity of spent coffee extracts by isother-
790 mal calorimetry. *J Therm Anal Cal*. 2018;132:1065–75.
- 791 48. Haman N, Longo E, Schiraldi A, Scampicchio M. Radical
792 scavenging activity of lipophilic antioxidants and extra-virgin
793 olive oil by isothermal calorimetry. *Thermochim Acta*.
794 2017;658:1–6.
- 795 49. Haman N, Romano A, Asaduzzaman M, Ferrentino G, Biasioli F,
796 Scampicchio M. A microcalorimetry study on the oxidation of
797 linoleic acid and the control of rancidity. *Talanta*.
798 2017;164:407–12.
- 799 50. Labuza TP, McNally L, Gallagher D, Hawkes J, Hurtado F.
800 Stability of intermediate moisture foods. I. Lipid oxidation.
801 *J Food Sci*. 1972;37:154–9.
- 802 51. Rahman MS, Labuza TP. Water activity and food preservation,
803 Handbook of Food Preservation, 2nd ed. (2007) M.S. Rahman,
804 Ed., CRC Press, Boca Raton, Florida, USA, 447–476.

- 805 52. Schiraldi A. The nature of biological systems as revealed thermal
806 methods“(2004) chap.2, D. Lorinczy Ed., Kluwer Academy
807 Publ., 31.
- 808 53. Schiraldi A. Microbial growth and metabolism: modelling and
809 calorimetric characterization. *Pure Appl Chem.* 1995;67:1873–8.
- 810 54. Fessas D, Schiraldi A. Isothermal calorimetry and microbial
811 growth: beyond modeling. *J Therm Anal Calorim.*
812 2017;130:567–72.
- 813 55. Riva M, Fessas D, Franzetti L, Schiraldi A. Calorimetric char-
814 acterization of different yeast strains in doughs. *J Therm Anal*
815 *Calorim.* 1998;52:753–64.
- 816 56. Gardikis K, Signorelli M, Ferrario C, Schiraldi A, Fortina MG,
817 Hatziantoniou S, Demetzos C, Fessas D. Microbial biosensors to
818 monitor the encapsulation effectiveness of Doxorubicin in chi-
819 meric advanced drug delivery nano systems: a calorimetric
820 approach. *Int J Pharm.* 2017;516:178–84.
- 821 57. Baranyi J, Pin C, Ross T. Validating and comparing predictive
822 models. *Int J Food Microbiol.* 1999;48:159–66.
- 823 58. Buchanan RL, Whiting RC, Damert WC. When is simple good
824 enough: a comparison of the Gompertz, Baranyi, and three-phase
linear models for fitting bacterial growth curves. *Food Microbiol.* 1997;14:313–26. 825
59. Peleg M. *Advanced quantitative microbiology for food and* 826
biosystems: models for predicting growth and inactivation. Boca 827
Raton: CRC Press; 2006. 828
60. Peleg M. *Microbial survival curves: interpretation, mathematical* 829
modeling and utilization. *Comments Theor Biol.* 830
2003;8(2003):357–87. 831
61. Schiraldi A. *Cell Dev Biol* 6 (2017) 185, [https://doi.org/10.4172/](https://doi.org/10.4172/2168-9296.1000185) 832
[2168-9296.1000185](https://doi.org/10.4172/2168-9296.1000185), and related appendix. 833
62. Schiraldi A. *Single Cell Biol.* 2017;6:166. <https://doi.org/10.4172/2168-9431.1000166>. 834
63. Schiraldi A. Data presented at MEDICTA 2017, Sept 24–27, 835
Loano (Italy). 836
64. Fessas D, Schiraldi A. Isothermal calorimetry and microbial 837
growth: beyond modeling. *J Therm Anal Cal.* 2017;130:567–72. 838
- 839 840

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