The Chemistry of Sourdough: Starters, Flours, Fermentation and Baking, and the Application of High-Field and Low-Field NMR Spectroscopy to the Starter, the Proof, the Loaf, and Their Extractable Components

A Process NMR Associates Technical White Paper

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A Global Phenomenon…. Stand Back World ….. Day Zero Sour Dough Research

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Abstract

Sourdough is a fermented cereal system in which lactic acid bacteria and yeasts, propagated in a flour–water matrix, transform the carbohydrate, protein, lipid, and mineral fractions of flour into a chemically distinct dough that, after baking, yields bread with characteristic acidity, aroma, texture, keeping quality, and nutritional attributes. This white paper presents a comprehensive treatment of the chemistry of sourdough from the perspective of an analytical laboratory equipped with both high-field solution and solid-state NMR instrumentation and low-field time-domain NMR relaxometry. The organisms of sourdough are described using the current taxonomy, in which the long-familiar *Lactobacillus sanfranciscensis* is now *Fructilactobacillus sanfranciscensis* and the yeast formerly known as *Kazachstania humilis* (earlier *Candida humilis* or *Candida milleri*) has been transferred to *Maudiozyma humilis*. The classification of sourdoughs into Types 0, I, II, III, and IV is explained and connected to the practical distinction between stiff and liquid starters, refreshment regimes, temperature, dough yield, and flour ash. The flours used for sourdough—bread wheat at several extraction rates, rye, durum, the hulled wheats spelt, einkorn, and emmer, barley, oat, maize, rice, sorghum, millet, teff, the pseudocereals buckwheat, quinoa, and amaranth, and legume flours—are compared in terms of the compositional attributes that govern fermentation: fermentable sugars and amylolytic potential, protein type and quantity, arabinoxylan and β-glucan content, fructans, phytate, lipids, endogenous enzymes, and buffering capacity. The core biochemistry is then developed in detail: maltose phosphorylase and the phosphoketolase pathway of heterofermentative lactobacilli, the use of fructose and oxygen as external electron acceptors with the resulting mannitol and acetate formation, the fermentation quotient, homofermentative metabolism, the yeast–bacterium partnership, acidification and buffering, cereal-protease-driven proteolysis and glutathione-reductase-mediated thiol exchange, arginine deiminase and ornithine formation, phytase activity, exopolysaccharide synthesis from sucrose, antifungal metabolites, and the fermentation-derived and thermally generated aroma compounds. The chemistry of the bread-making process itself—mixing, bulk fermentation, proofing, baking, cooling, and staling—is described and then contrasted point by point with straight-dough baker's-yeast bread, in which succinate rather than lactate and acetate is the dominant acid, the pH remains near 5.5, proteolysis is minimal, and firming during storage proceeds more rapidly. The second half of the paper reviews the use of NMR spectroscopy in sourdough science and constructs an analytical program suitable for a contract laboratory. High-field ^{1}H, ^{13}C, and two-dimensional NMR of aqueous and organic extracts are treated as the primary quantitative window on fermentation metabolites (maltose, glucose, fructose, lactate, acetate, ethanol, mannitol, glycerol, succinate, amino acids, ornithine, γ-aminobutyrate) and on flour-derived polymers (arabinoxylans, exopolysaccharides), ^{31}P NMR is presented as the definitive speciation method for phytate and its lower inositol phosphates, and diffusion-ordered spectroscopy is highlighted as a recent addition for following fermentation-induced changes in arabinoxylan size. High-resolution magic-angle spinning and ^{13}C cross-polarization solid-state NMR are described for intact flour, dough, and crumb, including the transformation of A-type starch crystallinity into amorphous gelatinized starch during baking and the return of double-helical order during staling, and for the hydration-dependent dynamics of gluten. Low-field time-domain relaxometry is developed at length because it is the technique most readily deployed at-line: the proton populations of dough and bread, their assignment to rigid biopolymer, exchangeable, and water-associated protons, the temperature-resolved changes accompanying gelatinization, the kinetics of staling, and the influence of sourdough acids and exopolysaccharides on water mobility. Magnetic resonance imaging of proofing, baking, and storage is reviewed. Chemometric treatment of NMR fingerprints is discussed together with the specific analytical problem of authenticating genuine sourdough bread against products acidified with added lactic and acetic acid, for which the co-metabolite pattern (mannitol, ornithine, ethanol residues, succinate, and sugar depletion) rather than acid content alone is argued to be the defensible criterion. A critical assessment of what NMR can and cannot resolve—volatile aroma compounds, lactate enantiomers, and gluten immunoreactivity among the latter—closes the technical review, and a proposed experimental program for Process NMR Associates is set out. Sixty references, each verified against the CrossRef registry, are cited.

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1. Introduction and Scope

Bread leavened by a spontaneously fermented, backslopped flour–water culture predates the isolation of pure baker's yeast by several millennia, and for most of the history of leavened bread the "sourdough" method was simply the method. The displacement of sourdough by compressed and later dried *Saccharomyces cerevisiae* during the nineteenth and twentieth centuries was driven by speed and reproducibility rather than by quality, and the current revival of sourdough baking—in artisan bakeries, in industrial plants that employ sourdough as a flavor and shelf-life ingredient, and in the home—has been accompanied by a substantial scientific literature that treats the fermented dough as a defined microbial ecosystem with a tractable biochemistry.[1–3]

The subject has attracted analytical chemists because sourdough is unusually rich in measurable chemistry. A single fermentation converts flour maltose into lactate, acetate, ethanol, carbon dioxide, and mannitol; releases and then consumes amino acids; hydrolyzes phytate; solubilizes and depolymerizes arabinoxylans; can synthesize exopolysaccharides from added sucrose; and generates a suite of antifungal and aroma-active molecules, all within a semi-solid, gas-filled, viscoelastic matrix whose water is partitioned among starch, gluten, pentosans, and solutes in ways that determine crumb texture and the rate at which the baked loaf stales.[2,4,5] Nuclear magnetic resonance is unusually well suited to this problem set because it spans the length and time scales involved. Solution-state high-field NMR of extracts quantifies the small-molecule metabolites and resolves the structures of polymers; ^{31}P NMR speciates the inositol phosphates; high-resolution magic-angle spinning and cross-polarization solid-state methods interrogate the intact dough and crumb; low-field time-domain relaxometry reports the mobility and partitioning of water and biopolymer protons in the raw dough, during baking, and throughout storage; and magnetic resonance imaging visualizes the gas cells whose growth during proofing and fixation during baking define the crumb.[6–8]

This white paper was prepared as a technical foundation for sourdough-related work at Process NMR Associates (PNA) and is intended to serve three purposes. First, it collects in one place the chemistry of sourdough starters, flours, and the bread-making process at a level of detail sufficient for the design of analytical studies. Second, it reviews what has been accomplished with NMR in this field, distinguishing established results from plausible extrapolations. Third, it proposes a concrete analytical program that draws on the high-field solution and solid-state capabilities and the low-field relaxometry instrumentation available at PNA, and it identifies the questions that such a program can be expected to answer as well as those it cannot. Throughout, the contrast between sourdough and conventional baker's-yeast bread is maintained because the majority of practical questions posed by bakers, ingredient suppliers, and regulators are comparative in nature: what does the sourdough process change, by how much, and how may the change be measured and documented.

The nomenclature of the organisms deserves a preliminary remark. The genus *Lactobacillus* was divided into twenty-five genera in 2020 on the basis of whole-genome phylogeny, so that the sourdough literature published before that date uses names that no longer correspond to the current taxonomy.[9] In this paper the current names are used, with the former name given at first mention; thus *Fructilactobacillus sanfranciscensis* (formerly *Lactobacillus sanfranciscensis*), *Limosilactobacillus reuteri*, *Limosilactobacillus fermentum*, *Limosilactobacillus pontis*, *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Companilactobacillus paralimentarius*, and *Furfurilactobacillus rossiae*. Among the yeasts, the organism isolated from San Francisco sourdough as *Torulopsis holmii* and later named *Candida milleri*, *Candida humilis*, and *Kazachstania humilis* has most recently been placed in the genus *Maudiozyma* as *Maudiozyma humilis*; the name *Kazachstania humilis* remains in wide use and is treated here as a synonym.

2. The Sourdough Ecosystem: Starters and Their Microbiota

2.1 Classification of Sourdoughs

A sourdough starter is a flour–water mixture in which a stable community of lactic acid bacteria (LAB) and, usually, yeasts has been established by repeated refreshment ("backslopping") with fresh flour and water. The classification that has become standard divides sourdoughs by process rather than by organism, because the process determines the organisms.[1,3] Type I sourdoughs are the traditional, continuously propagated starters, refreshed at intervals of several hours to a day, maintained at ambient temperature (typically 20–30 °C), and used at a stage of maturity at which the yeasts are still sufficiently active to leaven the final dough without added baker's yeast. Type II sourdoughs are industrial, generally liquid fermentations run at elevated temperature (30–45 °C) for two to five days, often with an inoculum of selected strains, in which the pH falls below 3.5 and the yeasts are largely absent or inactive, so that the product functions as an acidifier and flavor ingredient and the final dough is leavened with baker's yeast. Type III sourdoughs are dried preparations of Type II or Type I fermentations, dominated by organisms that survive drying, and are employed as acidifying and flavoring ingredients. Two further categories were introduced to accommodate practices that do not fit this scheme: Type 0 designates yeast-started pre-ferments such as the poolish, biga, and sponge, in which baker's yeast is the inoculum and any acidification is incidental; and Type IV designates hybrids in which a defined starter culture is combined with subsequent backslopping.[3] Table 1 summarizes the categories together with the process parameters and organisms that typify them.

Table 1. Classification of sourdough types by process and the microbiota that typically result. Ranges are indicative; individual bakeries and plants deviate substantially.

Type	Process description	Temperature and time	Dough yield (DY)	Typical pH at use	Characteristic organisms	Role in final dough
Type 0	Yeast-started pre-ferment (poolish, biga, sponge); no backslopping	20–28 °C, 2–16 h	150–250 (poolish ≈ 200)	5.0–5.6	*Saccharomyces cerevisiae* (baker's yeast); adventitious LAB at low numbers	Leavening and mild flavor development; not a sourdough in the strict sense
Type I	Traditional starter maintained by continuous backslopping; used at maturity for leavening	20–30 °C; refreshment every 4–24 h	150–250 (stiff levain/lievito madre ≈ 150–160; liquid levain ≈ 200+)	3.8–4.3	*Fructilactobacillus sanfranciscensis*, *Companilactobacillus paralimentarius*, *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Furfurilactobacillus rossiae*; yeasts *Maudiozyma humilis*, *S. cerevisiae*, *Kazachstania exigua*, *Torulaspora delbrueckii*, *Wickerhamomyces anomalus*	Leavening and acidification; no added baker's yeast required
Type II	Industrial liquid fermentation, frequently started with selected strains; long, warm, one-step	30–45 °C; 2–5 days	200–300	< 3.5	Acid- and heat-tolerant lactobacilli: *Limosilactobacillus reuteri*, *L. pontis*, *L. panis*, *L. fermentum*, *Lactobacillus amylovorus*; yeasts scarce	Acidifier and flavor ingredient; leavening supplied by baker's yeast
Type III	Dried (spray- or drum-dried) sourdough	Drying after Type I/II fermentation	Not applicable (powder)	Reconstituted 3.5–4.0	Drying-tolerant *L. plantarum*, *L. brevis*, *Pediococcus pentosaceus*	Convenience acidifier and flavoring; leavening by baker's yeast
Type IV	Defined starter culture followed by backslopping	As Type I or II	As Type I or II	3.6–4.2	Inoculated strains plus adventitious flour organisms that establish during propagation	Leavening and/or acidification depending on regime

The dough yield (DY), defined as the mass of dough obtained from 100 parts of flour, is the conventional measure of starter hydration. A DY of 150 corresponds to a stiff dough of 50 parts water per 100 parts flour; a DY of 200 corresponds to a batter of equal masses of flour and water; and a DY of 300 corresponds to a pourable slurry. The relationship between DY and chemistry is real and is discussed in Section 2.3, but it should be stated at the outset that the correlation between hydration and the lactate-to-acetate ratio, though often presented as a rule, is mediated by temperature, oxygen access, and flour composition and is not a fixed property of water content alone.

2.2 The Organisms and Their Metabolic Types

The LAB of sourdough belong overwhelmingly to the former genus *Lactobacillus* and to the genera *Pediococcus*, *Leuconostoc*, and *Weissella*.[1,10] What matters chemically is the fermentation type. Obligately heterofermentative lactobacilli—*F. sanfranciscensis*, *L. brevis*, *L. fermentum*, *L. reuteri*, *L. pontis*, and *F. rossiae* among the most frequent—metabolize hexoses through the phosphoketolase (6-phosphogluconate) pathway and produce lactate, carbon dioxide, and either ethanol or acetate in roughly equimolar amounts, the balance between ethanol and acetate depending on the availability of external electron acceptors. Facultatively heterofermentative species, of which *L. plantarum*, *C. paralimentarius*, and *Pediococcus pentosaceus* are examples, ferment hexoses homofermentatively to two moles of lactate per mole of hexose through the Embden–Meyerhof pathway, but can metabolize pentoses through phosphoketolase and produce acetate under those conditions. Obligately homofermentative species (*Lactobacillus amylovorus*, *Lactobacillus delbrueckii*) appear mainly in Type II fermentations. The distinction is the origin of the practical division of sourdoughs into those with relatively high acetate (heterofermentative, Type I) and those that are nearly pure lactic fermentations (homofermentative or Type II).[4]

The yeasts of sourdough are fewer in species but no less important. *Saccharomyces cerevisiae*, which is often introduced with flour handling or from the bakery environment, dominates in many Type I starters that are refreshed frequently and kept warm. *Maudiozyma humilis* and the closely related *Kazachstania exigua* dominate in traditional starters that are refreshed less often and are more acidic; these yeasts are notable for their tolerance of acetic acid at low pH and, in the case of *M. humilis*, for an inability to ferment maltose, a trait that underlies its stable association with maltose-fermenting *F. sanfranciscensis* (Section 4.2).[1,3] Other yeasts recovered with some regularity include *Torulaspora delbrueckii*, *Wickerhamomyces anomalus*, and *Pichia kudriavzevii*. The bacterial-to-yeast ratio in a mature Type I starter is typically of the order of 100:1, with LAB at 10^8–10^9 colony-forming units per gram and yeasts at 10^6–10^7.[1]

The classic account of the San Francisco process remains instructive. Kline and Sugihara isolated from the sourdough of that city a heterofermentative bacterium that fermented maltose in preference to glucose, produced lactic and acetic acid, and could not be assigned to any described species; they named it *Lactobacillus sanfrancisco*, and the companion paper of the same group identified the leavening yeast as a maltose-negative organism then classified as *Saccharomyces exiguus* (*Torulopsis holmii*), which was subsequently described as *Candida milleri* and is the organism now known as *M. humilis*.[11] The bacterium was subsequently renamed *Lactobacillus sanfranciscensis* and, after the 2020 reorganization, *Fructilactobacillus sanfranciscensis*, a name that draws attention to its preference for fructose as an electron acceptor.[9] Five decades of subsequent work have established that the *F. sanfranciscensis–M. humilis* association is the canonical Type I community in Europe and North America, although a large survey of five hundred home and bakery starters from four continents found *F. sanfranciscensis* in a majority but not all of them, found *S. cerevisiae* to be the most common yeast, and detected acetic acid bacteria (*Acetobacter*,

Gluconobacter) in a substantial minority, with acetic acid bacteria being associated with slower dough rise and distinct aroma profiles.[12] Geography, in that survey, was a weak predictor of community composition; the practices of the baker were stronger determinants. Table 2 lists the organisms most frequently encountered together with their fermentation type and the metabolites of analytical interest that each contributes.

Table 2. Principal sourdough organisms, their fermentation type, and the metabolites of analytical significance. Former names are given in parentheses for organisms renamed in the 2020 reorganization of *Lactobacillus*.

Organism (former name)	Fermentation type	Sourdough type in which most common	Principal metabolites and traits of analytical interest
Fructilactobacillus sanfranciscensis (*Lactobacillus sanfranciscensis*)	Obligately heterofermentative	Type I (wheat and rye)	Lactate, acetate, ethanol, CO_2; mannitol when fructose is present; maltose phosphorylase releases glucose; glutathione reductase drives thiol exchange in gluten; arginine deiminase yields ornithine
Levilactobacillus brevis (*L. brevis*)	Obligately heterofermentative	Type I, Type III	Lactate, acetate, ethanol, mannitol; pentose utilization; frequently γ-aminobutyrate (GABA) producer
Limosilactobacillus fermentum (*L. fermentum*)	Obligately heterofermentative	Type II, warm Type I	Lactate (DL), acetate, ethanol, mannitol; thermotolerant
Limosilactobacillus reuteri (*L. reuteri*)	Obligately heterofermentative	Type II	Lactate, acetate, ethanol, mannitol; reuteran and levan exopolysaccharides; glycerol conversion to 3-hydroxypropanal (reuterin) and 1,3-propanediol; produces reutericyclin in some strains
Limosilactobacillus pontis (*L. pontis*)	Obligately heterofermentative	Type II	Lactate, acetate, ethanol, mannitol; acid- and heat-tolerant
Furfurilactobacillus rossiae (*L. rossiae*)	Obligately heterofermentative	Type I	Lactate, acetate, ethanol; pentose utilization; vitamin B_{12} synthesis reported
Lactiplantibacillus plantarum (*L. plantarum*)	Facultatively heterofermentative	Type I, Type II, Type III, gluten-free sourdoughs	Predominantly lactate; phenyllactic and 4-hydroxyphenyllactic acids (antifungal); GABA; phenolic acid decarboxylation and reduction
Companilactobacillus paralimentarius (*L. paralimentarius*)	Facultatively heterofermentative	Type I	Predominantly lactate; frequent in Italian lievito madre
Lactobacillus amylovorus, *L. delbrueckii*	Obligately homofermentative	Type II	Lactate only; amylase activity in *L. amylovorus*
Pediococcus pentosaceus	Facultatively heterofermentative	Type I, Type III	Lactate (DL); survives drying
Leuconostoc citreum, *Weissella confusa*, *Weissella cibaria*	Obligately heterofermentative	Type I (especially non-wheat and legume sourdoughs)	Lactate, acetate, ethanol, mannitol; dextran (glucansucrase) from sucrose; low acidification
Maudiozyma humilis (*Kazachstania humilis*, *Candida humilis*, *Candida milleri*)	Yeast; fermentative	Type I (traditional, acidic, infrequently refreshed)	Ethanol, CO_2, glycerol; maltose-negative; acetate-tolerant at low pH

Organism (former name)	Fermentation type	Sourdough type in which most common	Principal metabolites and traits of analytical interest
Saccharomyces cerevisiae	Yeast; fermentative	Type 0, Type I (frequently refreshed), all baker's-yeast doughs	Ethanol, CO_2, glycerol, succinate; maltose-positive; invertase (fructan and sucrose hydrolysis)
Kazachstania exigua, *Torulaspora delbrueckii*, *Wickerhamomyces anomalus*, *Pichia kudriavzevii*	Yeasts; fermentative or oxidative	Type I	Ethanol, CO_2; ethyl acetate and other esters (*W. anomalus*); *P. kudriavzevii* acid-tolerant

2.3 Type I Starters: Stiff and Liquid Levains, Refreshment, and Temperature

The traditional Type I starter is managed by a small number of variables: the refreshment ratio (the mass of mature starter carried into each new batch of flour and water), the interval between refreshments, the hydration, the temperature, and the flour. Because the LAB and yeasts grow at different rates and respond differently to acid, each of these variables shifts the community and its products. The dependence of the growth rates of *F. sanfranciscensis* and *M. humilis* on temperature and pH has been modeled explicitly, with the bacterium having an optimum near 32–33 °C and the yeast an optimum near 28 °C and a considerably greater tolerance of low pH, so that warm propagation favors the bacterium relative to the yeast whereas cool propagation and infrequent refreshment favor the yeast.[1,10] These findings are the scientific basis of the craft baker's observation that a starter kept warm and refreshed often is more sour and rises more slowly, while a cool, well-fed starter rises vigorously and is milder.

Hydration exerts its effect principally through the concentration of solutes and the availability of oxygen. In a stiff starter (DY ≈ 150) solute concentrations and osmotic stress are higher, oxygen diffuses poorly, and the fermentation is more acidic at maturity in terms of titratable acidity; the lactic-to-acetic ratio in stiff, cool starters is frequently reported to be lower (that is, relatively more acetic acid) than in liquid, warm starters, an outcome usually attributed to the greater availability of oxygen and fructose as electron acceptors in the stirred or more dilute system and to the temperature dependence of the heterofermentative enzymes.[1,4] The Italian lievito madre, a stiff starter (DY ≈ 150–160) traditionally refreshed at intervals of a few hours at 26–30 °C and sometimes maintained under water or wrapped in cloth, represents one extreme; the French levain liquide (DY ≈ 200–250) kept at 25–30 °C in a stirred vessel represents the other. Both are Type I in the classification of Section 2.1 and both are dominated by *F. sanfranciscensis* and related organisms, but their fermentation quotients (Section 4.1) and their volatile profiles differ.

The flour used for refreshment matters as much as the regime. Whole-grain and rye flours introduce more fermentable sugar, more amylase, more phytate and phytase, more minerals and therefore more buffering capacity, and a larger and more diverse population of adventitious microorganisms than refined white flour. A starter refreshed with rye reaches a higher titratable acidity at the same pH than one refreshed with white wheat flour because the ash of rye buffers the medium; conversely, a starter converted from whole grain to white flour typically becomes less acidic and may lose part of its community.[2,3] These effects are chemically straightforward but are frequently misattributed by practitioners to changes in the "strength" of the culture.

2.4 Type II and Type III Starters and Defined Starter Cultures

Industrial sourdough production has different aims. A liquid Type II sourdough is fermented in a temperature-controlled tank, often inoculated with a selected strain or consortium, and run to a pH of 3.2–3.5 over two to five days at 30–45 °C. Under those conditions the acid- and heat-tolerant

Limosilactobacillus species (*L. reuteri*, *L. pontis*, *L. panis*, *L. fermentum*) and the homofermentative *L. amylovorus* dominate, yeasts are inhibited, and the product is a stable, pumpable acidifier that is dosed into the final dough at 5–20% of the flour weight.[3,10] The organisms of Type II sourdoughs have been described as having a lifestyle adapted to prolonged growth in a highly acidic, static environment, with adaptations that include acid tolerance mediated by the arginine deiminase and glutamate decarboxylase pathways; both pathways are of direct analytical interest because they yield ornithine and γ-aminobutyrate, respectively, which are quantifiable by NMR and serve as markers of Type II metabolism.[4,10] Drying a Type I or Type II sourdough to a Type III powder preserves acidity and flavor but selects for the organisms that tolerate drying, so that a Type III product is dominated by *L. plantarum*, *L. brevis*, and pediococci rather than by *F. sanfranciscensis*.

Commercial starter cultures, whether freeze-dried single strains or defined consortia, are widely used to initiate both Type I and Type II processes. Their contribution is reproducibility of acidification and, when selected for the purpose, specific functional traits: exopolysaccharide synthesis for texture, phytase for mineral release, antifungal metabolite production for shelf life, or a particular aroma profile. Whether a defined strain persists through subsequent backslopping is an ecological question and is not guaranteed; the adventitious flour community frequently displaces the inoculated strain within a few refreshment cycles unless the process parameters are held within the window in which the inoculated strain is competitive.[3] For an analytical laboratory the practical consequence is that the metabolite profile of a starter, not the identity of the culture that was purchased, is what must be measured.

2.5 Ecological and Chemical Drivers of Starter Composition

The parameters that govern the composition and stability of a sourdough community may be summarized as follows. Temperature selects between the bacterium and the yeast and among the bacteria (Section 2.3). Acidity, expressed as pH, selects for acid-tolerant organisms; the LAB drive the pH down and, in a stable Type I starter, the pH at maturity is typically 3.8–4.2 for wheat and 3.6–4.0 for rye, with titratable acidity of 8–15 mL of 0.1 mol L^{-1} NaOH per 10 g for white wheat starters and up to 20–25 mL for rye. Dough yield sets solute concentration and oxygen access. Flour composition sets the supply of maltose, sucrose, and fructose, the buffering capacity, and the load of endogenous enzymes. Salt, when added to a starter or to the final dough, inhibits the LAB more than the yeast and slows acidification. Oxygen, admitted by stirring or by the geometry of the vessel, is consumed by yeasts and by LAB that possess NADH oxidase and shifts the heterofermentative balance toward acetate.[1,3,13] These drivers are the levers by which a baker or a process engineer manipulates the chemistry of the product, and they are also the covariates that an analytical study must record if the metabolite data are to be interpretable.

3. Flours and Substrates

3.1 What the Flour Supplies

From the standpoint of the fermentation, a flour is a package of fermentable sugars, a source of further sugar through amylolysis, a protein fraction that may or may not form a gluten network, a set of cell-wall polysaccharides (arabinoxylans, β-glucans, cellulose) that bind water and are partly solubilized by acid and enzymes, a lipid fraction subject to enzymatic and oxidative change, a mineral fraction dominated by phytate-bound phosphorus that governs buffering capacity and mineral availability, and an inventory of endogenous enzymes—α- and β-amylases, proteases, phytase, xylanases, lipase, lipoxygenase, polyphenol oxidase—whose activities depend sharply on pH and are therefore switched on and off by the fermentation

itself.[2,14] The free sugars of white wheat flour are modest: maltose of the order of 1% together with sucrose at roughly 0.5% and small amounts of glucose, fructose, and raffinose, with the balance of the fermentable substrate being generated during the fermentation by β-amylase acting on damaged starch. The rate and extent of that amylolysis, which in turn depends on the damaged-starch content set by milling and on the pH trajectory, is one of the least appreciated determinants of sourdough behavior.

Table 3 collects the compositional attributes of the flours that are used, alone or in blends, for sourdough. The values are typical ranges assembled from the cereal chemistry literature and from commercial specifications; they are provided to guide expectations rather than to characterize any particular lot, and a laboratory undertaking a fermentation study should determine protein, ash, damaged starch, falling number, and free sugars on the actual flour used.

Table 3. Compositional attributes of flours used in sourdough baking that bear directly on the fermentation chemistry. Values are typical ranges on an as-is basis (approximately 12–14% moisture) and vary with cultivar, growing season, and milling; they are indicative, not specifications.

Flour	Protein (%)	Starch (%)	Gluten-forming capacity	Arabinoxylan (total / water-extractable, %)	β-Glucan (%)	Fructans (%)	Lipids (%)	Ash (%)	Phytate (%)	Endogenous enzymes and other traits relevant to sourdough
Bread wheat, white (≈ 72–75% extraction)	11–14	68–75	Strong; extensible	1.5–2.5 / 0.3–0.8	< 0.5	1–1.5	1–1.5	0.4–0.6	0.1–0.4	Moderate amylase (falling number 250–400 s); low phytase; low buffering; low microbial load
Bread wheat, whole grain	12–15	55–65	Strong but diluted by bran	5–7 / 0.5–1.0	0.5–1	1.5–2	2–3	1.5–1.8	0.6–1.2	Higher amylase and phytase; high buffering; bran particles disrupt gluten; richer adventitious microbiota
Durum (semolina and remilled semolina)	12–15	65–72	Strong, tenacious, less extensible	1.5–2.5 / 0.3–0.6	< 0.5	1–1.5	1.5–2	0.7–1.0	0.2–0.5	Carotenoid (lutein) pigments; traditional in southern Italian breads (Altamura) and Type I starters of the region
Spelt (*Triticum aestivum* subsp. *spelta*)	13–16	60–68	Moderate; extensible, less elastic	2–3 / 0.4–0.8	< 0.5	1–2	2–3	0.8–1.5 (whole)	0.3–0.8	Hulled wheat; high gliadin-to-glutenin ratio; sticky doughs; tolerates long, cool fermentation
Einkorn (*T. monococcum*)	15–18	55–65	Weak; poor gas retention	2–4 / 0.4–0.8	< 0.5	1–2	3–4	1.5–2.5 (whole)	0.5–1.0	High carotenoids and tocols; low loaf volume; strong reliance on acidification for crumb structure
Emmer (*T. dicoccum*)	13–17	58–66	Weak to moderate	2–4 / 0.4–0.8	< 0.5	1–2	2–3	1.5–2.5 (whole)	0.5–1.0	Hulled; intermediate between einkorn and durum

Flour	Protein (%)	Starch (%)	Gluten-forming capacity	Arabinoxylan (total / water-extractable, %)	β-Glucan (%)	Fructans (%)	Lipids (%)	Ash (%)	Phytate (%)	Endogenous enzymes and other traits relevant to sourdough
Rye, whole grain	8–12	55–65	Very poor (secalins); crumb held by pentosans and gelatinized starch	7–9 / 2–3	1–2	4–6	1.5–2	1.5–2.0	0.5–1.0	Highest cereal phytase; high α-amylase (low falling number); acidification obligatory to limit starch degradation; highest fructan of the common cereals
Barley, whole grain (hull-less)	10–12	55–65	None (hordeins)	4–6 / 0.5–1.0	4–7	0.5–1.5	2–3	1.5–2.5	0.5–1.0	β-Glucan raises viscosity and water binding; used at 10–30% in composite breads
Oat, whole grain	12–16	50–60	None (avenins)	2–4 / 0.3–0.5	3–6	0.5–1	5–9	1.5–2.5	0.6–1.2	Active lipase and lipoxygenase (heat treatment usual); β-glucan; low fermentable sugar
Maize (corn)	8–10	70–75	None (zein)	3–6 / < 0.5	< 0.5	< 0.5	4–5 (whole)	1–1.5	0.5–1.0	Gluten-free; low water absorption; requires hydrocolloid or exopolysaccharide for crumb
Rice (white)	6–8	75–80	None	< 1 / < 0.2	< 0.2	< 0.2	0.5–1	0.3–0.6	< 0.2	Gluten-free base flour; very low buffering; rapid pH fall
Sorghum, whole grain	10–12	65–75	None (kafirins)	3–5 / < 0.5	< 0.5	< 0.5	3–4	1.5–2	0.5–1.0	Phenolic acids and (in some cultivars) condensed tannins; traditional African sourdoughs; kafirins resistant to proteolysis
Millet (pearl, finger) and teff	7–12	60–70	None	2–5 / < 0.5	< 0.5	< 0.5	2–5	1.5–3	0.3–0.8	Teff is the substrate of injera (spontaneous multi-day sourdough); high iron and calcium in teff and finger millet
Buckwheat	12–13	55–65	None (globulins)	1–3 / < 0.5	< 0.5	< 0.5	2–3	1.5–2.5	0.8–1.5	Pseudocereal; rutin and quercetin; small starch granules; distinct flavor
Quinoa and amaranth	13–16	55–65	None	1–3 / < 0.5	< 0.5	< 0.5	5–7	2–3	0.5–1.5	Pseudocereals; high lysine; saponins in unwashed quinoa; very small starch granules

Flour	Protein (%)	Starch (%)	Gluten-forming capacity	Arabinoxylan (total / water-extractable, %)	β-Glucan (%)	Fructans (%)	Lipids (%)	Ash (%)	Phytate (%)	Endogenous enzymes and other traits relevant to sourdough
Legume flours (chickpea, faba, lentil, pea, red bean)	18–26	40–50	None	1–3 / < 0.5	< 0.5	< 0.5	1–6	2.5–3.5	0.5–1.5	Raffinose-family oligosaccharides 2–6%; lipoxygenase (beany notes); trypsin inhibitors; *Weissella*-dominated sourdoughs with dextran synthesis are used to improve texture

3.2 Bread Wheat at Different Extraction Rates

Refined white flour is the reference substrate of most of the mechanistic literature because it is the cleanest system: fermentable sugar is limited, amylase activity is moderate, buffering is low, and the gluten network is strong enough to retain gas without help from the fermentation. The pH of a white-flour Type I sourdough falls to about 4.0 within 12–24 h and the titratable acidity is comparatively low. Increasing the extraction rate—that is, retaining more of the bran and germ—raises the ash and therefore the buffering capacity, so that a larger quantity of acid must be produced to reach the same pH; it also raises the amylase and phytase activities, the phytate content, the arabinoxylan content, the lipid content (with the attendant risk of lipoxygenase-driven oxidation), and the inoculum of adventitious microorganisms carried on the bran.[2,14] A whole-wheat sourdough is therefore more acidic in absolute terms, ferments faster, and undergoes a considerably more extensive degradation of phytate than a white sourdough held under the same regime. The presence of bran also interferes mechanically with gluten development, which is one of the reasons that whole-grain sourdough breads are denser; part of the benefit of sourdough in whole-grain baking is that acidification softens the bran and solubilizes arabinoxylans, which improves the water-holding of the crumb and partly compensates for the disruption of the gluten network.[5]

3.3 Rye

Rye is the flour for which sourdough is not optional but chemically necessary. Rye proteins (secalins) do not form a gluten network of any consequence, so the crumb of a rye bread is held together by a gel of gelatinized starch and arabinoxylans rather than by protein. Rye also carries a high α-amylase activity that, in an unacidified dough, would degrade the starch during the early stages of baking (when the temperature has risen enough to gelatinize the starch but not yet enough to inactivate the enzyme) and produce a gummy, collapsed crumb. Acidification to a pH near 4.0–4.3 in the dough reduces the amylase activity into a tolerable range and, at the same time, solubilizes the pentosans, increases water binding, and imparts the sour flavor that is traditional in rye breads.[1,5] Rye also has the highest phytase activity and among the highest phytate and fructan contents of the common cereals, so that a rye sourdough shows the most complete phytate hydrolysis and the most extensive fructan degradation. Because rye flour buffers strongly, a rye sourdough of pH 3.7 may have twice the titratable acidity of a wheat sourdough of the same pH; expressed as lactate and acetate concentrations by NMR, the difference is immediately apparent.

3.4 Durum and the Hulled Wheats

Durum semolina, the flour of southern Italian and some Mediterranean breads, forms a strong but relatively inextensible gluten and yields a dense yellow crumb; its traditional Type I starters are among the best characterized in the Italian literature, and the breads have been studied by high-resolution magic-angle spinning NMR and isotope-ratio mass spectrometry as a problem in origin and identity (Section 8.1).[15] Spelt, einkorn, and emmer—hulled wheats that have regained commercial interest—differ from bread wheat chiefly in their gluten. Spelt gluten is extensible but weak in elastic recovery and tends to produce sticky, slack doughs that benefit from the strengthening effect of a moderate acidification and from shorter mixing. Einkorn gluten is so weak that gas retention is poor by any process; einkorn sourdoughs succeed as flatbreads or as dense loaves in which the acid-solubilized pentosans and the acid-induced changes in protein solubility, rather than an elastic gluten network, provide what structure there is. All three hulled wheats are richer in lipids, carotenoids, and protein than bread wheat, and their higher protein contents translate into more extensive proteolysis and higher free amino acid concentrations in the fermented dough.

3.5 Other Cereals, Pseudocereals, and Legume Flours

The remaining flours are gluten-free, and sourdough fermentation has been examined as a means of improving the texture, flavor, and shelf life of gluten-free breads that otherwise depend on starches and hydrocolloids.[16] The chemical logic is that the acidification, the in situ synthesis of exopolysaccharides by strains of *Weissella* and *Leuconostoc* in the presence of added sucrose, and the solubilization of cell-wall polysaccharides can partly substitute for the structuring function of gluten. Barley and oat contribute β-glucans, which raise dough viscosity and water binding; oat brings in a lipid fraction of 5–9% with active lipase, so that heat-treated oat flour is normally used. Maize, rice, sorghum, millet, and teff have low protein functionality and low buffering, and their sourdoughs acidify rapidly; sorghum in particular contains phenolic acids and, in some cultivars, condensed tannins that interact with proteins and can inhibit the LAB. Teff is the substrate of injera, a spontaneously fermented Ethiopian flatbread whose multi-day fermentation is one of the oldest continuously practiced sourdough processes. Buckwheat, quinoa, and amaranth are pseudocereals with globulin rather than prolamin storage proteins, small starch granules, and characteristic minor components (rutin in buckwheat, saponins in quinoa). Legume flours—chickpea, faba bean, lentil, pea, and red bean—supply 18–26% protein, C-type starch, and 2–6% raffinose-family oligosaccharides, which are α-galactosides that are fermented by the microbiota with a reduction in the flatulence-associated oligosaccharides and an accompanying release of galactose; NMR-based metabolomics has been applied to the fermentation of bean flour by defined LAB and yeasts, and it discriminated homo- and heterofermentative metabolism, genus, and the accumulation of free amino acids including γ-aminobutyrate.[17] Sourdough fermentation of legume flours is also a route to reducing the beany off-flavors that arise from lipoxygenase action on linoleic and linolenic acids, because the acidification and the reducing conditions established by the LAB limit the enzyme.

4. The Chemistry of Sourdough Fermentation

4.1 Carbohydrate Metabolism of the Lactic Acid Bacteria

The dominant fermentable substrate of a wheat or rye sourdough is maltose, supplied initially by the flour and continuously by β-amylase acting on damaged starch, with sucrose, glucose, and fructose as minor substrates.[4] The heterofermentative sourdough lactobacilli, of which *F. sanfranciscensis* is the archetype, take up maltose and cleave it with maltose phosphorylase rather than with a hydrolytic maltase (Equation 1).

This is a consequential detail. Phosphorolysis yields one glucose-1-phosphate, which enters the phosphoketolase pathway without expenditure of ATP, and one free glucose, which the organism does not immediately use and which is largely excreted into the dough while maltose remains available. The excreted glucose supports the growth of maltose-negative yeasts such as *M. humilis*, which is the biochemical basis of the celebrated *F. sanfranciscensis–M. humilis* mutualism; the yeast in turn does not compete for maltose and supplies the bacterium with amino acids and vitamins released by autolysis, and its consumption of glucose relieves the bacterium of the glucose-mediated repression of maltose uptake that occurs in many other lactobacilli.[1,4]

$$\text{Maltose} + P_i \rightarrow \beta\text{-D-glucose-1-phosphate} + \text{D-glucose} \quad \text{(maltose phosphorylase)} \tag{1}$$

The phosphoketolase pathway oxidizes glucose-6-phosphate to 6-phosphogluconate and then to ribulose-5-phosphate with the loss of one carbon as CO_2 and the generation of two NADH. Xylulose-5-phosphate is cleaved by phosphoketolase to glyceraldehyde-3-phosphate and acetyl phosphate. Glyceraldehyde-3-phosphate proceeds through the lower half of glycolysis to pyruvate and lactate with no net change in the NADH balance, whereas the acetyl phosphate must be disposed of in a manner that regenerates the two NAD^+ consumed in the oxidative steps. In the absence of an external electron acceptor the acetyl phosphate is reduced through acetyl-CoA and acetaldehyde to ethanol, consuming the two NADH, so that the overall stoichiometry is that of Equation 2: one lactate, one ethanol, and one CO_2 per hexose, with one ATP. When an external electron acceptor is present, the acetyl phosphate is instead converted to acetate by acetate kinase with the formation of a second ATP, and the two NADH are re-oxidized by reduction of the acceptor.[4,13] In sourdough the physiologically dominant acceptor is fructose, which is reduced to mannitol by mannitol dehydrogenase (Equation 3); oxygen, reduced to water or hydrogen peroxide by NADH oxidase, is the other acceptor of practical importance, and citrate and certain aldehydes can serve in some species.[13] The organism gains energy by using the acceptor, and *F. sanfranciscensis* has been shown to grow faster and reach a higher cell density when fructose is available.

$$C_6H_{12}O_6 \rightarrow CH_3CH(OH)COO^- + H^+ + CH_3CH_2OH + CO_2 \quad \text{(heterofermentation, no external acceptor)} \tag{2}$$

$$C_6H_{12}O_6 + 2\ C_6H_{12}O_6\ \text{(fructose)} \rightarrow CH_3CH(OH)COO^- + CH_3COO^- + 2\ H^+ + CO_2 + 2\ C_6H_{14}O_6\ \text{(mannitol)} \quad \text{(heterofermentation with fructose as acceptor)} \tag{3}$$

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH(OH)COO^- + 2\ H^+ \quad \text{(homofermentation)} \tag{4}$$

Equation 3 has three analytical consequences. First, the acetate content of a heterofermentative sourdough is set by the supply of electron acceptor rather than by the supply of sugar, and the addition of sucrose (a source of fructose after cleavage by invertase or by the glucansucrases of Section 4.6) or of fructose raises acetate and lowers the fermentation quotient. Second, mannitol accumulates stoichiometrically with the acetate formed by this route and is therefore a specific and quantifiable marker of heterofermentative metabolism operating with fructose as acceptor; a sourdough with substantial acetate but no mannitol has obtained its acetate from another acceptor (oxygen, in a stirred liquid starter) or from acetic acid bacteria, or the acetate was added. Third, the mannitol is a sugar alcohol that persists through baking, is sweet, and is itself a fermentable polyol that is counted among the FODMAPs (Section 4.9). Facultatively heterofermentative species such as *L. plantarum* produce two moles of lactate per hexose by the Embden–Meyerhof pathway (Equation 4) and produce acetate only from pentoses or under specific conditions; a sourdough dominated by such organisms is a nearly pure lactic fermentation with a high fermentation quotient and little mannitol. Figure 1 summarizes the pathways.

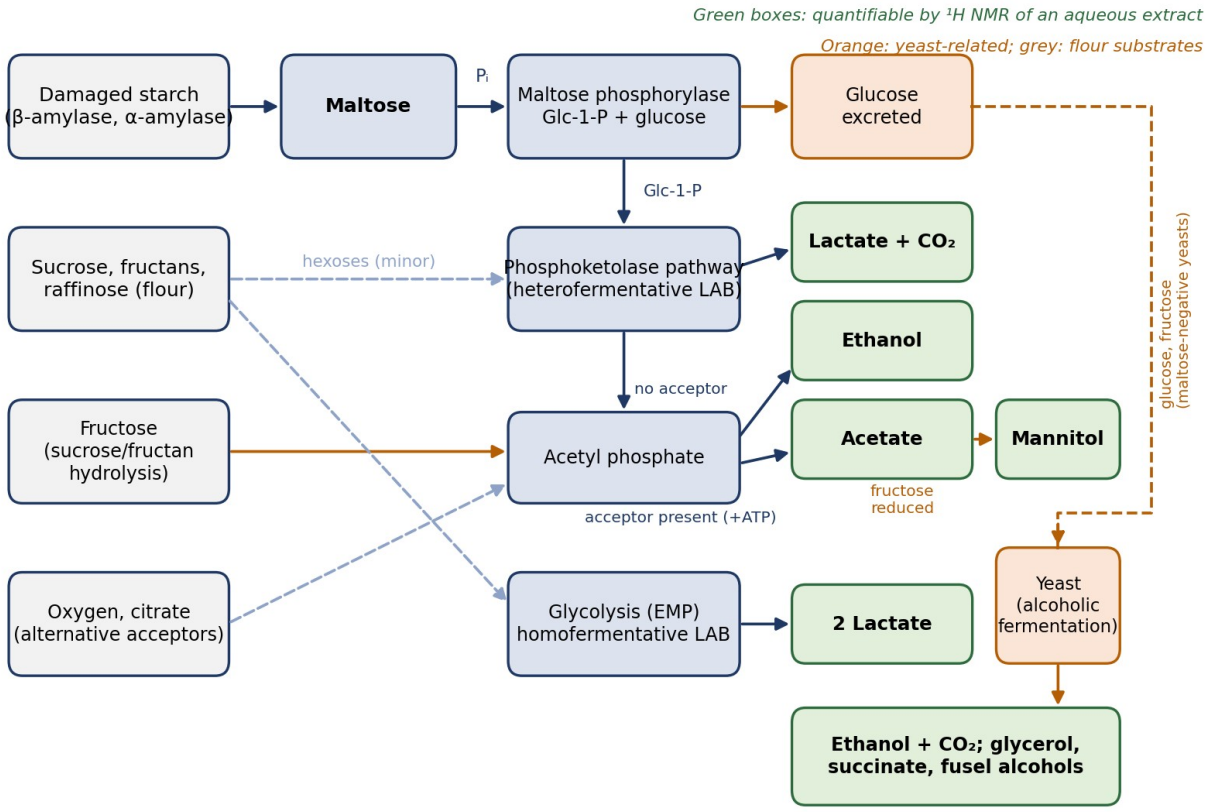

Figure 1. Schematic of the carbohydrate metabolism that generates the principal sourdough metabolites. Heterofermentative lactobacilli cleave maltose by phosphorolysis, excrete glucose (supporting maltose-negative yeasts), and dispose of acetyl phosphate either as ethanol (no external acceptor) or as acetate with reduction of fructose to mannitol. Facultatively heterofermentative and homofermentative species convert hexoses to lactate alone. Yeasts convert hexoses to ethanol, CO_2, glycerol, and succinate. Every species in the boxes at the right is directly quantifiable by ¹H NMR of an aqueous extract. Schematic prepared for this white paper; not drawn from measured data.

The fermentation quotient (FQ), defined as the molar ratio of lactate to acetate (Equation 5), is the conventional descriptor of the acid balance. Values in the range of roughly 2 to 4 are commonly quoted as sensorially optimal for wheat sourdough bread, higher values yielding a flat, purely lactic sourness and lower values a sharp, vinegar-like character; rye breads are traditionally made with more acetic acid. Because lactate and acetate are among the best-resolved signals in a ¹H NMR spectrum of a dough extract (Section 7.2), FQ is one of the quantities most naturally determined by NMR. The dough yield (Equation 6) is required for converting concentrations expressed per unit mass of dough into concentrations per unit mass of flour, which is the basis on which fermentation data are normally compared.[1,4]

$$FQ = n(\text{lactate}) / n(\text{acetate}) = [c(\text{lactate}) / 90.08] / [c(\text{acetate}) / 60.05], \text{ with c in g kg}^{-1} \quad (5)$$

$$DY = 100 \times (m_flour + m_water) / m_flour \quad (6)$$

A worked illustration fixes the magnitudes. A mature white-wheat Type I sourdough of DY 200 typically contains 8–12 g of lactic acid and 2–4 g of acetic acid per kilogram of dough. Taking 10.0 g kg^{-1} and 3.0 g kg^{-1}, the molar concentrations are 111 and 50 mmol kg^{-1} and FQ = 2.2. Expressed per kilogram of flour (DY 200), these are 20 and 6 g kg^{-1}. A rye sourdough at DY 180 may contain 15–25 g kg^{-1} of lactic acid and 4–8 g kg^{-1} of acetic acid; a Type II wheat sourdough fermented with *L. reuteri* at 40 °C for 48 h may exceed 20 g kg^{-1} of lactic acid with an FQ above 4. The mannitol accompanying the acetate of Equation 3 in the

first example, if fructose were the sole acceptor, would be about 9 g kg^{-1} of dough (50 mmol kg^{-1} × 182.2 g mol^{-1}), a quantity that is readily quantified by NMR and that is characteristically absent from a bread acidified with purchased acids.

4.2 The Yeasts and the Lactic Acid Bacterium–Yeast Partnership

The yeasts of sourdough convert hexoses to ethanol and carbon dioxide by alcoholic fermentation (Equation 7), with glycerol as the principal by-product of redox balancing under the osmotic stress of the dough and with succinate, acetate, and a spectrum of higher alcohols and esters as minor products.[1,18] The leavening of a Type I sourdough bread is almost entirely the work of the yeasts; the LAB produce CO_2 by the phosphoketolase pathway, but at a rate that is small compared with that of an active yeast population, and the contribution of bacterial CO_2 to loaf volume is minor. Yeast metabolism also consumes free amino acids for growth, so that the amino acid pool of a mixed sourdough reflects the balance between release by proteolysis and consumption by the yeasts.[19]

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2 \quad \text{(alcoholic fermentation; minor products glycerol, succinate, acetate)} \tag{7}$$

The partnership between *F. sanfranciscensis* and *M. humilis* is the textbook case of a stable, non-competitive association. The bacterium consumes maltose and excretes glucose; the yeast, being maltose-negative, consumes the glucose and does not compete for maltose; the yeast is unusually tolerant of the acetic acid that the bacterium produces at low pH, whereas *S. cerevisiae* is inhibited under the same conditions; and the yeast supplies amino acids, peptides, and vitamins on autolysis.[1,4] The result is a community that reproduces itself through indefinite backslopping under the conditions of a traditional bakery. Where *S. cerevisiae* is the yeast instead—as in many frequently refreshed home starters and in all baker's-yeast doughs—the maltose is shared, the ethanol is higher, and the community is less stable under acid stress, so that a starter of this kind that is neglected tends to drift toward a more acidic, yeast-poor state.

4.3 Acidification, Buffering, and the Speciation of the Acids

The pH of a sourdough is the outcome of acid production against the buffering capacity of the flour, which resides in its proteins, phosphates, and minerals. White wheat flour buffers weakly and a sourdough made from it reaches pH 3.9–4.2 at titratable acidities of 8–15 mL of 0.1 mol L^{-1} NaOH per 10 g; rye flour buffers strongly and reaches pH 3.6–3.9 at titratable acidities of 15–25 mL per 10 g. The pH governs the speciation of the acids, and the speciation governs their sensory and antimicrobial effect. Lactic acid (pK_a 3.86) and acetic acid (pK_a 4.76) are both largely protonated at sourdough pH: at pH 3.8 approximately 53% of lactic acid and 90% of acetic acid are undissociated, whereas at the pH of 5.5 that characterizes a baker's-yeast dough the undissociated fractions are 2% and 15%, respectively. Since it is the undissociated acid that crosses microbial membranes, inhibits molds and rope-forming bacilli, and is perceived as sourness, the antifungal and sensory potency of a given quantity of acetic acid is roughly six times greater at sourdough pH than at yeast-dough pH. The same speciation determines the chemical shifts of the acid resonances in the ^{1}H NMR spectrum of an extract (Section 7.1), which is the reason that extracts must be measured at a fixed pH.

The acids also act directly on the flour polymers. Below pH 4.5 the solubility of gluten proteins increases, the electrostatic repulsion between protonated glutenin chains loosens the network, and dough strength decreases; below pH 4.0 the cereal aspartic proteases become active (Section 4.4). Acid solubilizes arabinoxylans and thereby increases the water-binding capacity of the dough, an effect that is most pronounced in rye and whole-grain systems. Acid also inhibits α-amylase, which is favorable in rye and in

high-amylase wheat flours but which, in a low-amylase white flour, can limit the supply of maltose to the point that a very acidic starter becomes sugar-starved.[2,5] The net effect on the final dough is a softer, more extensible, somewhat stickier mass that develops faster and tolerates less mixing than a yeast dough.

4.4 Proteolysis, Thiol Exchange, and Amino Acid Metabolism

Proteolysis in sourdough is primarily the work of cereal enzymes, not of the bacteria. Wheat and rye contain aspartic proteases with pH optima in the region of 3–4.5 that are essentially inactive in a yeast dough at pH 5.5 and are switched on by sourdough acidification; carboxypeptidases and aminopeptidases of the grain complete the hydrolysis of the resulting peptides to free amino acids.[19,20] The sourdough LAB contribute little extracellular proteolytic activity toward gluten but possess intracellular peptidases that hydrolyze the transported peptides, and they contribute decisively in a second way: the heterofermentative lactobacilli, *F. sanfranciscensis* in particular, possess glutathione reductase and reduce the oxidized glutathione of the flour to reduced glutathione, which participates in thiol–disulfide exchange with the intermolecular disulfide bonds of the glutenin macropolymer. The result is a depolymerization of the largest glutenin aggregates that is independent of hydrolysis and that softens the dough, increases the solubility of the gluten proteins, and increases the accessibility of the proteins to the cereal proteases.[4,20] A sourdough prepared with a heterofermentative strain therefore differs from a chemically acidified dough at the same pH: the acid alone activates the proteases, but the thiol exchange adds a specific and substantial depolymerization.

The free amino acid pool of a mature wheat sourdough is several times that of the unfermented dough and is dominated by the amino acids abundant in gluten—glutamine and glutamate, proline, leucine, phenylalanine—together with those liberated by the specific bacterial pathways. Arginine is converted by the arginine deiminase pathway of many heterofermentative lactobacilli through citrulline to ornithine, with the release of ammonia and the generation of ATP; the ammonia contributes to acid tolerance and the ornithine, which accumulates in the dough because it is not consumed by the yeast, is the precursor of 2-acetyl-1-pyrroline, the roasty, cracker-like key odorant of wheat bread crust, which is formed during baking from ornithine and 2-oxopropanal.[19,21] Glutamate is decarboxylated by the glutamate decarboxylase of strains of *L. brevis*, *L. plantarum*, and *L. reuteri* to γ-aminobutyrate (GABA), a second acid-tolerance mechanism whose product is retained in the bread and has been the object of functional-food claims. Yeasts consume amino acids and, through the Ehrlich pathway, convert leucine, isoleucine, valine, and phenylalanine to 3-methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-1-propanol, and 2-phenylethanol, which are among the dominant volatile alcohols of fermented dough.[18,21] All of the non-volatile species in this paragraph—glutamate, proline, leucine, phenylalanine, ornithine, citrulline, GABA, and the branched-chain amino acids—give resolved resonances in the ^{1}H NMR spectrum of a dough extract and are quantifiable by NMR at the concentrations (0.1–5 mmol kg^{-1}) at which they occur.

Gluten degradation by sourdough deserves a specific caution. The proteolysis and thiol exchange described above modify the gluten network and reduce its molecular weight distribution, and selected consortia of lactobacilli combined with fungal proteases under prolonged fermentation have been shown to hydrolyze gluten to concentrations below 10 mg kg^{-1}, at which the product was tolerated by celiac subjects in a clinical challenge.[22] That outcome required specifically selected strains, exogenous fungal proteases, a semi-liquid fermentation of long duration, and a formulation designed for the purpose; it is not a property of ordinary sourdough. A conventional sourdough bread retains the large majority of its immunoreactive gluten, and NMR, which reports on soluble small molecules and on the bulk dynamics of the protein, does not measure immunoreactive gluten and cannot substitute for the ELISA or mass-spectrometric methods on which any gluten-related claim must rest.

4.5 Enzymatic Conversions of the Flour Constituents

The pH trajectory of a sourdough sequentially switches the endogenous enzymes of the flour. β-Amylase, the enzyme responsible for maltose supply from damaged starch, has its optimum near pH 5–5.5 and remains active, though diminishing, through the early hours of the fermentation; α-amylase is progressively inhibited below pH 4.5. Phytase, the enzyme that hydrolyzes myo-inositol hexakisphosphate (phytate, IP_6) stepwise to the lower inositol phosphates and inorganic phosphate, has an optimum near pH 5–5.5 in wheat and rye, and it has been demonstrated that the moderate acidification produced by sourdough is sufficient to activate the endogenous enzyme and to reduce the phytate content of whole-wheat dough substantially, whereas a yeast dough at pH 5.5–6.0 leaves most of the phytate intact.[23] The contribution of the LAB themselves to phytate hydrolysis is small in comparison with that of the cereal phytase; when the endogenous enzyme was heat-inactivated, fermentation by LAB alone degraded little phytate, and across wheat, rye, and oat the extent of degradation tracked the endogenous phytase activity of the flour rather than the bacterial count.[24] ^{31}P NMR is the natural technique for following this chemistry and is treated in Section 7.5.[25]

The cell-wall polysaccharides are modified by acid and by enzymes of both cereal and microbial origin. Water-unextractable arabinoxylan is partly solubilized, and the water-extractable arabinoxylan is progressively reduced in molecular size during the fermentation; the latter process has been followed directly by high-temperature diffusion-ordered ^{1}H NMR spectroscopy, which showed rising self-diffusion coefficients and transverse relaxation times of the water-extractable arabinoxylans with fermentation time in wheat sourdoughs fermented with several LAB strains (Section 7.4).[26] Lipids are hydrolyzed by cereal lipase to free fatty acids, which are then subject to lipoxygenase-catalyzed oxidation to hydroperoxides and, by cleavage, to the aldehydes (hexanal, (E)-2-nonenal) that contribute both to bread aroma and to off-flavors; sourdough conditions moderate this chemistry because the acidity and the reducing environment limit lipoxygenase, and certain lactobacilli additionally convert unsaturated fatty acids to hydroxy fatty acids with antifungal activity.[2] Phenolic acids, chiefly ferulic acid bound to arabinoxylan, are released by esterases and, in *L. plantarum* and related species, decarboxylated to 4-vinylguaiacol or reduced to dihydroferulic acid; the former is a clove-like odorant of some breads.[2] These lipid- and phenolic-derived species occur at concentrations that place most of them below the practical limit of quantification of NMR in a crude extract, and their study remains the province of chromatography.

4.6 Exopolysaccharides

Several sourdough LAB synthesize homopolysaccharides from sucrose by extracellular glucansucrases and fructansucrases. Glucansucrases transfer the glucosyl moiety of sucrose to a growing α-glucan chain and release fructose (Equation 8); the products are dextrans (α-(1→6) backbones with α-(1→3) or α-(1→2) branches) in *Weissella* and *Leuconostoc*, reuteran (a mixed α-(1→4)/α-(1→6) glucan) in *L. reuteri*, and mutan in some streptococci. Fructansucrases produce levan (β-(2→6)) or inulin-type fructans and release glucose.[27] The fructose released by glucansucrases is the electron acceptor of Equation 3, so that the in situ synthesis of dextran in a sourdough by a heterofermentative organism is inseparable from an increase in acetate and mannitol; the acetate is the price of the exopolysaccharide.

$$\text{n Sucrose} \rightarrow (\text{Glucose})_n + \text{n Fructose} \quad \text{(glucansucrase)};\ \text{n Sucrose} \rightarrow (\text{Fructose})_n + \text{n Glucose} \quad \text{(fructansucrase)} \tag{8}$$

The in situ production of *Weissella confusa* dextran in wheat sourdough at concentrations of the order of 1–2% of flour weight has been shown to increase the viscosity of the sourdough and, in the bread, to increase loaf volume, reduce crumb hardness, and retard staling, effects that are attributable to the water-binding and

network-forming properties of a high-molecular-weight, largely linear α-(1→6)-glucan and to the concurrent acidification.[27,28] The structure of these dextrans—the backbone linkage, the degree and type of branching, and the molecular weight—is determined by NMR, and the ^{1}H and ^{13}C spectra of the dextrans produced by *Leuconostoc citreum* and *W. confusa* have been assigned in detail; the *W. confusa* dextran is a nearly linear α-(1→6)-glucan with a few percent of α-(1→3)-linked branches (Section 7.4).[29] Glucansucrases also catalyze acceptor reactions in which maltose and other sugars serve as acceptors of the glucosyl unit, yielding gluco-oligosaccharides (panose and isomalto-oligosaccharides) that are prebiotic and that appear in the NMR spectrum of the extract as an additional family of α-(1→6) anomeric signals. Exopolysaccharide synthesis is one of the few mechanisms by which a sourdough can be made to improve the volume and softness of a bread, as opposed to merely its flavor and keeping quality, and it is the mechanism most heavily exploited in gluten-free and legume-flour sourdoughs.

4.7 Antifungal and Preservative Metabolites

The extended mold-free shelf life of sourdough bread is the joint result of the undissociated organic acids at crumb pH (Section 4.3) and of a set of specific antifungal metabolites. Phenyllactic acid and 4-hydroxyphenyllactic acid, produced by *L. plantarum* from phenylalanine and tyrosine, were the first such compounds isolated from a sourdough organism, and their structures were established by NMR and mass spectrometry in the original work.[30] Cyclic dipeptides, hydroxy fatty acids formed from linoleic acid by certain lactobacilli, and the reuterin (3-hydroxypropanal) formed by *L. reuteri* from glycerol add to the inventory.[2] Acetic acid is nevertheless the antifungal agent that matters quantitatively in most sourdough breads, and the relationship between the acetate concentration of the dough, the crumb pH, and the mold-free shelf life is the practical target of most industrial sourdough formulation. Propionic acid, which is added as calcium propionate to conventional bread and is produced in only trace amounts by sourdough LAB, is quantifiable by ^{1}H NMR after steam distillation of the crumb, and a validated method for propionic and sorbic acid in bread has been reported; the same approach can distinguish a sourdough bread preserved by its own acids from a bread that has been dosed with propionate.[31]

4.8 Flavor Chemistry

The flavor of sourdough bread is assembled from three sources: the non-volatile acids and their salts, which supply sourness and, in the case of acetate, a pungency that is perceived retronasally; the volatile products of the fermentation; and the volatiles generated thermally in the crust. The fermentation volatiles of a sourdough differ from those of a yeast dough principally in the higher concentrations of acetic acid and of the diacetyl, acetoin, and 2,3-butanediol that arise from pyruvate metabolism in the LAB, and in the balance of the yeast-derived Ehrlich alcohols (3-methyl-1-butanol, 2-phenylethanol) and their acetate esters, which depends on the yeast species and on the availability of the parent amino acids.[21] Heterofermentative sourdoughs are richer in acetic acid and ethyl acetate; homofermentative and Type II sourdoughs are richer in diacetyl and in the lactic-acid-dominated sourness. The crust flavor is generated by the Maillard reaction and Strecker degradation from the amino acids and reducing sugars of the dough surface: 2-acetyl-1-pyrroline from ornithine, 3-methylbutanal from leucine, methional from methionine, 2-furanmethanol and furfural from sugar dehydration, and 4-vinylguaiacol from ferulic acid. Because the sourdough fermentation raises the concentrations of the amino acid precursors and specifically of ornithine, the crust of a sourdough bread has been shown to contain more 2-acetyl-1-pyrroline and to score higher in roasty character than the crust of a yeast bread from the same flour, whereas the crumb of the sourdough bread is distinguished by acetic acid, 3-methyl-1-butanol, and 2-phenylethanol.[19,21] The odorants of bread occur at microgram-per-kilogram concentrations that are three to five orders of magnitude below the quantification limit of NMR in

any realistic extract; their study requires gas chromatography–olfactometry and stable-isotope-dilution mass spectrometry. What NMR measures, and measures well, are the non-volatile precursors—the amino acids, ornithine, the sugars, and the acids—whose concentrations at the end of fermentation predict the thermal flavor chemistry of the crust.

4.9 Nutritional Consequences and the Limits of the Claims

Four nutritional effects of sourdough fermentation are well established. The hydrolysis of phytate improves the availability of magnesium, iron, and zinc, the effect being greatest in whole-grain and rye breads and dependent on the extent of acidification and the fermentation time (Sections 4.5 and 7.5).[23,24] The presence of lactic acid in the bread lowers the postprandial glucose and insulin responses relative to a yeast bread of the same composition, an effect attributed to a reduced rate of starch digestion arising from acid-induced interactions between starch and gluten during baking; acetic acid acts through a different mechanism involving gastric emptying.[32] Fructans—the principal FODMAP of wheat and rye—are hydrolyzed during fermentation by the invertase of *S. cerevisiae* and by the fructanases of certain lactobacilli, and sourdough breads of substantially reduced fructan content can be produced; the same fermentation, however, generates mannitol through Equation 3, and because mannitol is a polyol FODMAP, a heterofermentative sourdough can lower fructans while raising polyols, so that the net FODMAP outcome depends on the organisms and the conditions and cannot be assumed.[33] Finally, the extended shelf life reduces the need for chemical preservatives. Beyond these, claims that sourdough bread is "gluten-free," that it is suitable for celiac subjects, or that it contains meaningful quantities of probiotic organisms (the LAB do not survive baking) are not supported, and an analytical laboratory that is asked to substantiate such claims should decline to do so on the basis of metabolite data. What NMR contributes to the nutritional questions is quantitative: the residual phytate and inositol phosphate profile by ^{31}P NMR, the lactate and acetate concentrations by ^{1}H NMR, and the fructan, mannitol, glucose, and fructose balance by ^{1}H NMR, each of which is an objective descriptor of the bread that can be reported without recourse to health claims.

5. From Dough to Loaf: The Chemistry of the Bread-Making Process

5.1 Mixing and the Formation of the Gluten Network

Mixing hydrates the flour and converts a dispersion of protein bodies and starch granules into a continuous viscoelastic network. In wheat, the storage proteins—monomeric gliadins and polymeric glutenins linked by intermolecular disulfide bonds through their high- and low-molecular-weight subunits—absorb water, are stretched and aligned by the mechanical work, and are reorganized by thiol–disulfide interchange into a network whose elasticity resides in the glutenin polymers and whose extensibility is conferred by the gliadins.[14,34] The molecular basis of the elasticity has been described by the loop-and-train model, in which the glutamine-rich repetitive domains of the high-molecular-weight glutenin subunits associate through interchain hydrogen bonds in "train" regions and are separated by hydrated, mobile "loop" regions; extension converts loops into trains and the restoring force arises from the re-formation of the loops, and the model was developed in significant part from solid-state NMR and infrared observations of the hydration-dependent mobility of the subunits.[35] Water is distributed among the flour polymers in proportions that have long been estimated, for a conventional dough, as roughly half to the starch, a third to the gluten, and the remainder to the pentosans, the last of which absorb many times their own weight; damaged starch, which is produced during milling, absorbs several times more water than intact granules and is also the substrate of the β-amylase that supplies maltose to the fermentation. Mixing also occludes air, and the bubbles so

entrained are the nuclei of all the gas cells of the finished crumb, since carbon dioxide generated during fermentation does not nucleate new bubbles in a dough but diffuses into the existing ones.[36,37]

In a conventional straight dough the network is strengthened by oxidation: ascorbic acid is oxidized to dehydroascorbic acid, which oxidizes the reduced glutathione of the flour and thereby prevents that glutathione from depolymerizing the glutenin by thiol exchange; the net effect is a larger glutenin macropolymer and a stronger, less extensible dough. The sourdough process pushes in the opposite direction. The reducing conditions established by the heterofermentative LAB, the glutathione reductase of *F. sanfranciscensis*, and the acid activation of the cereal proteases all reduce the size of the glutenin macropolymer, and a dough into which a substantial proportion of mature sourdough has been incorporated is softer, more extensible, more prone to stickiness, and quicker to reach its optimum development than the same flour mixed with yeast alone.[5,20] Practical consequences follow: sourdoughs are mixed for shorter times and at lower intensity, the proportion of pre-fermented flour is limited (commonly 10–30% of the total flour for wheat breads and higher for rye), and the strengthening additives of industrial baking are usually omitted or reduced.

5.2 Bulk Fermentation and Proofing

During bulk fermentation and proofing the gas produced by the yeasts dissolves in the aqueous phase of the dough until saturation is reached and then diffuses into the entrained gas nuclei, which expand against the viscoelastic resistance of the gluten–starch matrix. The dough rises, the gas cells enlarge and become polydisperse, and the thin liquid films (lamellae) that separate adjacent cells become progressively more important to gas retention as the cell walls thin; the stability of these films is controlled by the surface-active lipids and proteins of the flour, and the effect of these minor components on bubble growth has been examined directly by magnetic resonance imaging (Section 10).[37] The rate of gas production is set by the yeast population, its physiological state, the temperature, and the sugar supply, and the rate of gas retention is set by the rheology of the network. A Type I sourdough leavened without added baker's yeast carries a yeast population that is one to two orders of magnitude smaller than that of a yeast dough, and its proof is correspondingly longer—commonly 3–8 h at 24–28 °C or 8–18 h with cold retardation at 4–8 °C, as against 1–2 h for a straight dough—so that the acidification, proteolysis, and enzymatic conversions of Section 4 proceed in the final dough as well as in the starter.[1] Cold retardation is widely used in artisan sourdough baking precisely because it decouples gas production, which slows sharply at refrigerator temperature, from the enzymatic and flavor chemistry, which continues, and it yields a more acidic and more aromatic bread than a warm proof of equal gas production.

The acids accumulating during the final proof continue to soften the network, so that a sourdough is at once more extensible and less elastic than a yeast dough at the same stage; the gas cells of a sourdough are on average larger and more irregular, and the crumb more open, than those of a yeast bread from the same flour, a difference that is exaggerated by the high hydration favored in contemporary artisan practice. The concentration of ethanol in the dough at the end of proof is of the order of 0.5–2% by mass in both systems and is largely lost during baking; the concentrations of lactate, acetate, mannitol, and amino acids at the end of proof, by contrast, are retained in the crumb, and it is at this stage that an extract taken for NMR analysis is most informative about what the fermentation has done (Section 7.3).

5.3 Baking

Baking converts the foam of the proofed dough into the sponge of the crumb through a sequence of physical and chemical events that occur as the temperature front advances from the surface inward. In the first

minutes the thermal expansion of the gas, the release of dissolved CO_2, the vaporization of ethanol and water, and the final burst of yeast activity produce the oven spring; the yeasts are killed near 55 °C. Between about 60 and 75 °C the starch granules gelatinize in the limited water available, swelling, losing their crystalline order, and leaching amylose, while the gluten proteins denature and aggregate through heat-induced disulfide cross-linking, so that the continuous phase changes from a viscoelastic gluten network filled with starch granules to a rigid composite of swollen, partly fused starch granules embedded in a coagulated protein matrix.[14,38] It is in this temperature window, before the enzymes are inactivated near 75–90 °C, that α-amylase acts on the gelatinizing starch to produce dextrins and maltose; the amount of this hydrolysis is small in a well-acidified sourdough, moderate in a yeast dough, and, in an unacidified rye dough, sufficient to ruin the crumb. The rupture of the thinnest cell walls as the gas expands connects the cells and converts the closed-cell foam into the open-cell sponge; the crumb temperature then plateaus near 98 °C as long as water remains to evaporate. At the surface, where the water content falls and the temperature exceeds 120 °C, the Maillard reaction and caramelization produce the color and the crust aroma from the reducing sugars and amino acids of the dough surface, with the ornithine-dependent formation of 2-acetyl-1-pyrroline as the signature reaction of the sourdough crust (Section 4.8).[21]

Baking also changes the analyte inventory that survives to the finished bread. Lactic acid is non-volatile and is retained quantitatively in the crumb, so that the lactate content of a bread is a direct record of the fermentation; acetic acid is volatile and is partly lost, more from the crust than from the crumb, so that the fermentation quotient of a bread is higher than that of its dough; ethanol is almost entirely driven off; mannitol, glycerol, succinate, and the amino acids are retained; the reducing sugars and amino acids of the crust are consumed; and the exopolysaccharides and arabinoxylans are retained but are incorporated into the gelatinized starch–protein matrix. The residual water content of the crumb, typically 38–45%, and its distribution among the biopolymers, which is what the low-field NMR of Section 9 measures, are fixed at this stage and then evolve during storage.

5.4 Cooling and Staling

On cooling, the leached amylose retrogrades within hours into a network of double helices that sets the crumb and gives the fresh bread its resilience; the amylopectin of the swollen granules then recrystallizes over a period of days into double-helical, B-type ordered domains, and this slow amylopectin retrogradation, together with the redistribution of water from crumb to crust and among the crumb biopolymers, is the principal molecular basis of the firming and loss of resilience that constitute staling.[38] The crumb firms even in hermetically sealed loaves that lose no water, which demonstrates that staling is not simply drying; it is reversed transiently by reheating above the melting temperature of the retrograded amylopectin, which demonstrates that the recrystallization is the reversible component. The role of the gluten is more subtle: the migration of water from gluten to starch as the amylopectin crystallizes, and the consequent stiffening of the protein phase, have been argued from combined relaxometry, calorimetric, and texture data to contribute to firming beyond the contribution of the starch crystallization itself (Section 9.4).[39]

Sourdough retards staling by several routes: the acid-activated α-amylase and the bacterial enzymes generate low-molecular-weight dextrins that interfere with amylopectin recrystallization; the solubilized arabinoxylans and any exopolysaccharides bind water and slow its migration; the acid-modified gluten holds more water; and the lower pH itself has been reported to slow the rate of starch recrystallization.[5,27] The magnitude of the effect depends on the organisms, the degree of acidification, and the proportion of sourdough in the formula, and it is not universal—an over-acidified sourdough with excessive proteolysis can produce a bread that is soft when fresh but crumbly and dry on storage. Because the staling process is a

change in the ordering of starch and in the partitioning of water, it is measured with unusual directness by the solid-state and low-field NMR methods of Sections 8 and 9.

5.5 Sourdough Bread Contrasted with Baker's-Yeast Bread

Conventional non-sour bread is leavened with *S. cerevisiae* added at 1–3% of flour weight, either in a straight-dough process (all ingredients mixed at once, bulk fermentation of 1–3 h, proof of about 1 h), a sponge-and-dough process (a portion of the flour pre-fermented with yeast for several hours as a Type 0 sponge, then mixed with the remaining ingredients), or a no-time process such as the Chorleywood bread process, in which intense mechanical development, ascorbic acid, fat, and enzymes replace bulk fermentation altogether.[14,18] The pH of the dough remains in the range 5.3–5.8. Succinate, produced by the yeast through a branch of the tricarboxylic acid cycle and from glutamate, is the acid that accounts for most of the modest fall in pH of a yeast dough, with acetate and lactate present at much lower concentrations than in a sourdough; the succinate singlet near δ 2.4 ppm in the ^{1}H NMR spectrum of a bread extract is, accordingly, a marker of yeast metabolism, whereas the lactate doublet and the acetate singlet are markers of bacterial metabolism.[18] Proteolysis is minimal at pH 5.5, the glutenin macropolymer is preserved or, with oxidants, enlarged, phytate is largely retained in whole-grain formulations, fructans are partly hydrolyzed by the yeast invertase, mannitol is absent, and the crumb pH of 5.3–5.7 requires a preservative such as calcium propionate if a mold-free shelf life beyond a few days is to be achieved. Table 4 sets the two systems side by side. Industrial practice increasingly blends them: a Type II or Type III sourdough is dosed into a yeast-leavened dough at 5–20% of flour weight for flavor and shelf life, and the resulting bread is intermediate in every parameter of the table. A laboratory asked whether a commercial "sourdough" bread was leavened by a Type I culture, acidified with a Type II or III ingredient, or acidified with purchased lactic and acetic acid, is being asked a question that the metabolite pattern of Section 7.7 is designed to answer.

Table 4. Chemical and process contrasts between Type I sourdough bread and conventional baker's-yeast (straight-dough) bread from the same wheat flour. Values are typical ranges; hybrid processes fall between the columns.

Attribute	Type I sourdough bread	Baker's-yeast (straight-dough) bread
Leavening agent	Yeasts of the starter (*M. humilis*, *S. cerevisiae*) at 10^6–10^7 CFU g^{-1}; LAB at 10^8–10^9 CFU g^{-1}	Added *S. cerevisiae* at 1–3% of flour, 10^8–10^9 CFU g^{-1}; adventitious LAB negligible
Pre-ferment	Backslopped starter, 10–30% of flour pre-fermented (higher for rye)	None, or a yeast sponge/poolish (Type 0)
Total fermentation time	8–30 h including starter maturation and cold retardation	1.5–4 h; effectively zero in no-time processes
Dough pH at end of proof	3.8–4.5	5.3–5.8
Titratable acidity (mL 0.1 M NaOH per 10 g crumb)	4–9 (wheat), 8–16 (rye)	1–3
Principal acids	Lactic (5–15 g kg^{-1} crumb), acetic (1–4 g kg^{-1}); FQ 2–5 in bread	Succinic (0.5–2 g kg^{-1}), traces of acetic and lactic
Ethanol at end of proof	0.5–2%, largely lost on baking	1–2%, largely lost on baking
Mannitol	1–10 g kg^{-1} when heterofermentative LAB and fructose/sucrose are present	Absent
Glycerol	Present (yeast), 1–3 g kg^{-1}	Present (yeast), 1–3 g kg^{-1}
Free amino acids	Elevated several-fold; ornithine, GABA present	Low; consumed by yeast; ornithine and GABA negligible

Attribute	Type I sourdough bread	Baker's-yeast (straight-dough) bread
Gluten macropolymer	Depolymerized by thiol exchange and acid proteolysis; softer, more extensible dough	Preserved or enlarged by oxidants; stronger, more elastic dough
Amylase activity during baking	Suppressed by low pH; limited dextrin formation	Active until inactivation; moderate dextrin formation; rye unusable without acidification
Phytate (whole grain)	50–100% hydrolyzed depending on time and pH	Largely retained (< 30% hydrolyzed)
Fructans	Substantially reduced (yeast invertase and LAB fructanase)	Partly reduced (yeast invertase)
Crust key odorant 2-acetyl-1-pyrroline	Higher (ornithine precursor)	Lower
Crumb volatiles	Acetic acid, ethyl acetate, 3-methyl-1-butanol, 2-phenylethanol, diacetyl	3-Methyl-1-butanol, 2-phenylethanol, ethanol; little acetic acid
Crumb pH	4.2–4.8	5.3–5.7
Mold-free shelf life without preservative	Extended (acetic and phenyllactic acids at low pH)	Short; calcium propionate commonly added
Staling rate	Retarded (dextrins, solubilized pentosans, exopolysaccharides, modified gluten)	Faster unless anti-staling enzymes or emulsifiers are added
Typical additives	None	Ascorbic acid, enzymes (amylase, xylanase), emulsifiers (DATEM, SSL), calcium propionate
Loaf volume and crumb	Often lower volume; open, irregular, chewy crumb	Higher volume; fine, uniform, soft crumb
Glycemic response	Lower (lactic acid effect)	Reference

6. NMR Spectroscopy in Sourdough Science: Overview and Method Selection

Nuclear magnetic resonance contributes to sourdough science through five distinct modalities, each addressing a different physical state of the sample and a different set of questions, and the first task in designing a study is to match the modality to the question. High-field solution-state NMR (7 T and above; 300 MHz and above for ^{1}H) of aqueous and organic extracts provides the quantitative inventory of small-molecule metabolites, the structural characterization of soluble polymers, and the speciation of phosphorus; it is the modality with the largest information content per experiment and the one on which chemometric fingerprinting is built.[6,40] High-resolution magic-angle spinning (HR-MAS) ^{1}H NMR interrogates the mobile components of an intact flour, dough, or crumb sample without extraction, at the cost of broader lines and a bias toward the mobile fraction.[15] Solid-state ^{13}C cross-polarization magic-angle spinning (CP/MAS) NMR reports on the ordered and amorphous states of starch and on the hydration-dependent dynamics of gluten in the intact solid, and it is the technique that follows the loss of crystallinity on baking and its return during staling.[41,42] Low-field time-domain NMR relaxometry (0.5–1.5 T; 20–60 MHz), which does not resolve chemical shifts, measures the transverse and longitudinal relaxation of the proton populations of dough and bread and thereby the mobility and partitioning of water among the biopolymers; it is rapid, non-destructive, inexpensive, and suitable for at-line use, and it is the technique of choice for following mixing, proofing, baking, and staling in the intact material.[7,43] Magnetic resonance imaging (MRI), at high or low field, visualizes the spatial distribution of the gas cells and the water in dough and

bread and is the only NMR method that reports on the structure of the crumb at the length scale of the cell.[8,36] Benchtop high-resolution NMR (1–2 T permanent magnets; 60–100 MHz for ^{1}H) occupies an intermediate position: it resolves the major metabolites in an extract with reduced resolution and sensitivity and brings quantitative spectroscopy to the bakery or the pilot plant.[44] Table 5 maps these modalities to the sample states and questions of sourdough science and to the key literature.

Table 5. NMR modalities applicable to sourdough science, the sample state each addresses, the information obtained, and representative literature.

Modality (typical field)	Sample state	Information obtained	Principal sourdough and bread questions addressed	Representative references
High-field solution ^{1}H NMR (7–14 T)	Aqueous extract of flour, starter, dough, or crumb; D_2O, buffered	Identity and concentration of sugars, organic acids, alcohols, polyols, amino acids, nucleosides, betaine, choline; fingerprint for chemometrics	Fermentation kinetics; FQ; mannitol/ornithine/GABA markers; authentication; flour origin	Lamanna et al. (durum origin); Tatulli et al. (fermented legume flour); Scharinger et al. (preservatives in bread)
High-field ^{13}C, 2D (COSY, TOCSY, HSQC, HMBC) and DOSY NMR	Aqueous extract; isolated exopolysaccharide or arabinoxylan	Structure of dextrans, levans, arabinoxylans; branching; linkage; molecular-size distribution by diffusion	Exopolysaccharide characterization; fermentation-induced changes in arabinoxylan size; confirmation of metabolite identity	Maina et al. (dextran/levan assignment); Weckx et al. (DOSY of arabinoxylans during sourdough fermentation); Lavermicocca et al. (antifungal structure)
^{31}P NMR (7–14 T)	Alkaline aqueous extract of flour, dough, or crumb	Speciation and quantification of IP_6 and lower inositol phosphates, orthophosphate, phospholipids (in organic extracts)	Phytate degradation by cereal, yeast, and LAB phytases; mineral availability	Reale et al. (^{31}P NMR of wholemeal dough fermentation); Kemme et al. (inositol phosphate quantification)
HR-MAS ^{1}H NMR (9–14 T, 4–6 kHz spinning)	Intact flour, dough, crumb (30–50 mg in rotor)	Mobile small molecules and mobile polymer segments in the intact matrix; qualitative to semi-quantitative	Rapid characterization and classification of flours and breads without extraction	Brescia et al. (flours and breads of southern Italy)
Solid-state ^{13}C CP/MAS NMR (7–14 T)	Flour, dough, crumb, crust (dried or native moisture)	Crystalline vs amorphous starch; double-helix content; A/B/V polymorphs; gluten segmental dynamics via CP kinetics and ^{1}H relaxation	Gelatinization on baking; retrogradation on staling; crust crystallinity; gluten hydration; effect of acidification and exopolysaccharides on starch order	Gidley and Bociek; Morgan et al.; Baik et al.; Primo-Martín et al.; Calucci et al.; Nowacka-Perrin et al.
Low-field TD-NMR relaxometry (0.5–1.5 T; 20–60 MHz)	Intact dough, crumb, model systems; 5–10 g samples	FID and CPMG proton populations, T_2 and T_1 distributions; temperature-resolved changes	Water distribution in dough; mixing; proofing; gelatinization; staling kinetics; effects of acids, pentosans, exopolysaccharides	Ruan et al.; Doona and Baik; Bosmans et al.; Curti et al.; Engelsen et al.; Rondeau-Mouro et al.; Tang et al.
Multinuclear relaxation (^{2}H, ^{17}O)	Deuterated or ^{17}O-enriched dough and bread	Water-specific mobility free of exchange complications	Water binding during staling	Kim-Shin et al.
MRI and MR microscopy (0.2–11 T)	Whole dough piece, loaf, slice	Gas cell size distribution and growth; porosity; water distribution and migration; local expansion during baking	Proofing kinetics; effect of ingredients on bubble growth; baking; crumb–crust water migration on storage	van Duynhoven et al.; Rouillé et al.; Bajd and Serša; Wagner et al.; Lodi et al.
Benchtop high-resolution ^{1}H NMR (1–2 T; 60–100 MHz)	Aqueous extract, minimal preparation	Lactate, acetate, ethanol, succinate, glycerol, major sugars; mannitol with deconvolution	At-line fermentation monitoring; FQ; quality control of starters and Type II sourdoughs	Blümich (compact NMR review)

Several general points about the application of NMR to sourdough should be stated before the modalities are treated individually. NMR is inherently quantitative when the experiment is run under conditions of complete relaxation and uniform excitation, and it requires no analyte-specific calibration; a single internal standard of known concentration calibrates every resolved resonance in the spectrum, and this is the principal reason that a laboratory equipped with high-field NMR can produce a complete inventory of the non-volatile metabolites of a sourdough in one experiment where chromatography would require several.[40] NMR is comparatively insensitive, with practical limits of quantification in a crude extract of the order of 10–50 µmol L^{-1} at 400–600 MHz and 0.5–1 mmol L^{-1} at 60–80 MHz, so that the odor-active volatiles, the antifungal metabolites other than acetate, and the phenolic derivatives of Section 4.5 lie outside its reach. NMR is blind to chirality in an achiral solvent, so that D- and L-lactate, whose ratio is a species-dependent fingerprint of the LAB (most sourdough lactobacilli, including *F. sanfranciscensis* and *L. plantarum*, produce DL-lactate in species-specific proportions, *Leuconostoc* and *Weissella* produce D-lactate, and yeasts and mammals produce L-lactate), are not distinguished without a chiral shift reagent or derivatization; enzymatic assays or chiral chromatography remain the methods of choice for the enantiomeric ratio. And NMR of extracts reports on what was extracted: the choice of solvent, pH, and clean-up determines which of the analytes of Sections 4 and 5 appear in the spectrum, and the extraction must be designed with the question in view.

7. High-Field Solution-State NMR of Extracts

7.1 Extraction and Sample Preparation

The quality of a solution-state NMR study of sourdough is decided at the extraction step, because the dough is a living system whose composition continues to change during sample handling, because the extract contains polymers that broaden the baseline and bind small molecules, and because the chemical shifts of the acids and amino acids depend on pH. The following protocol has been assembled from the practices reported in the cited metabolomic and quantitative studies of flours, fermented flours, and breads and from the beverage qNMR practice of the author's laboratory, and it is offered as a defensible starting point rather than a validated method.[17,31,40,45]

Flour is extracted by suspending 1.00 g in 10.0 mL of water or of the D_2O phosphate buffer described below, shaking for 30 min at room temperature, and centrifuging at 10,000 g for 10 min; the supernatant contains the free sugars (sucrose, raffinose, glucose, fructose, and a little maltose), the free amino acids, betaine and choline, the organic acids of the grain (malate, citrate, and small amounts of succinate and fumarate), the water-soluble arabinoxylans, and the albumins and globulins. Starter and dough are sampled by weighing 2.00 g into a tube containing 8.0 mL of ice-cold water, homogenizing for 1 min, shaking for 15 min in an ice bath, and centrifuging at 4 °C; the low temperature suppresses the amylolytic and fermentative activity that would otherwise continue to generate maltose and consume it during the extraction. The supernatant is passed through a 0.45 µm filter and then through a 3 kDa ultrafiltration membrane, which removes the proteins, the polysaccharides, and the exopolysaccharides and yields an extract with a flat baseline; where the polymers are themselves the analytes (Section 7.4), a portion of the filtered but not ultrafiltered supernatant is retained. Crumb and crust are separated, the crumb is crumbled, and 5.00 g are extracted with 20.0 mL of water in the same way; bread extracts contain soluble dextrins and gelatinized starch fragments that produce broad envelopes between δ 3.4 and 4.0 ppm and near δ 5.4 ppm, and ultrafiltration is essential for quantitative work on the small molecules. A lyophilized starter or crumb may be extracted at 100–200 mg mL^{-1} where the sensitivity of a benchtop instrument requires higher concentration.

The NMR sample is prepared by combining 540 µL of extract with 60 µL of a D_2O solution containing the internal standard and buffer, so that the final sample is 10% D_2O for the field-frequency lock. Two buffering strategies are in use and serve different purposes. For metabolomic profiling the sample is brought to pH 7.40 ± 0.05 with a phosphate buffer of high ionic strength (final concentration 100–150 mmol L^{-1}), which places lactate, acetate, succinate, and the amino acids in their fully deprotonated forms and holds their chemical shifts within ±0.005 ppm across samples of differing native acidity, a precondition for the spectral alignment on which multivariate analysis depends. For targeted quantification of the organic acids alone, the sample may instead be clamped at a fixed low pH near 2.5–3.0, at which the acids are fully protonated, their methyl resonances are shifted downfield by 0.08–0.25 ppm (Table 6) into regions that are frequently less congested, and the risk of citrate and succinate overlapping with the glutamine and glutamate multiplets is reduced. Whichever strategy is chosen, it must be applied identically to every sample in a set, and the pH of each NMR sample should be recorded after addition of the buffer. The internal standard for ultrafiltered extracts is 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4) at 0.5–1.0 mmol L^{-1}; in unfiltered extracts TSP binds to proteins and its integral is attenuated, and 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS-d_6) or an electronic reference (ERETIC or PULCON) is preferable. Maleic acid, which has served the author's laboratory well in beverages, is usable at fixed low pH but its singlet is pH-sensitive and it should not be used with the neutral-buffer protocol. All extracts are analyzed within 24 h of preparation or stored at −80 °C.

7.2 The ^{1}H NMR Spectrum of a Sourdough Extract

A 400–600 MHz ^{1}H NMR spectrum of a buffered, ultrafiltered extract of a mature wheat sourdough resolves, without further separation, the fermentable sugars and their depletion, the three fermentation products that define the process, the polyols produced by the yeasts and by heterofermentative LAB, the organic acids of yeast metabolism, the free amino acids released by proteolysis and the amino acid derivatives produced by the LAB, the quaternary ammonium compounds of the grain, and, in the aromatic region, the aromatic amino acids, the nucleosides released by yeast autolysis, and formate. Table 6 lists the resonances on which quantification is normally based, with chemical shifts at pH 7.4 and, for the acids, at pH 2.5–3.0, together with the multiplicities and coupling constants that identify them and notes on the overlaps that must be managed. The values are drawn from the standard aqueous-metabolite databases and from the cited studies of fermented flours and bread, and they are given to ±0.02 ppm; the shifts of the amino acids and of citrate, succinate, and malate are pH-dependent between pH 3 and 7 and will differ from the tabulated values in an unbuffered sample.

Table 6. Diagnostic ^{1}H NMR resonances of the metabolites of sourdough and bread extracts in D_2O-containing aqueous solution at 298 K, referenced to TSP-d_4 at 0.00 ppm. Chemical shifts are given at pH 7.4 (phosphate buffer) and, where materially different, at pH 2.5–3.0 for the fully protonated acids. Multiplicities: s singlet, d doublet, t triplet, q quartet, dd doublet of doublets, m multiplet. Quantification notes identify the resonance normally integrated and the overlaps to be managed.

Compound	Resonance (pH 7.4), δ ppm, multiplicity, J	At pH 2.5–3.0	Protons	Origin in sourdough	Quantification notes
Lactate	1.33 (d, 6.9 Hz) CH_3; 4.11 (q) CH	1.41 (d); 4.36 (q)	3; 1	LAB (homo- and heterofermentative)	CH_3 doublet; threonine CH_3 (1.33 d) overlaps at pH 7.4 and is resolved at low pH; D/L not distinguished
Acetate	1.92 (s) CH_3	2.08 (s)	3	Heterofermentative LAB (with electron acceptor); yeast (minor); added vinegar	Singlet; check for overlap with GABA/glutamate multiplets at 1.9–2.1 at pH 7.4; resolved at low pH

Compound	Resonance (pH 7.4), δ ppm, multiplicity, J	At pH 2.5–3.0	Protons	Origin in sourdough	Quantification notes
Ethanol	1.18 (t, 7.1 Hz) CH_3; 3.66 (q) CH_2	unchanged	3; 2	Yeast; heterofermentative LAB without acceptor	CH_3 triplet; largely lost from bread on baking
Succinate	2.41 (s) CH_2	2.67 (s)	4	Yeast (TCA branch, glutamate); marker of yeast metabolism	Singlet; near glutamine/glutamate γ-CH_2 (2.45/2.34) at pH 7.4
Glycerol	3.56, 3.65 (dd) CH_2; 3.78 (m) CH	unchanged	4; 1	Yeast (redox balance); heterofermentative LAB	Congested region; use 3.56 dd with deconvolution or 2D
Mannitol	3.67 (dd), 3.75 (m), 3.80 (m), 3.87 (dd)	unchanged	8 (CH)	Heterofermentative LAB with fructose acceptor; absent in yeast bread	Congested with sugars; quantify by 3.87 dd with deconvolution, by ^{13}C (δ 64.4, 70.3, 72.1), or by HSQC; diagnostic authentication marker
Formate	8.46 (s) CH	8.26 (s)	1	LAB pyruvate–formate lyase; heat	Clean singlet in aromatic region
Maltose	5.41 (d, 3.9 Hz) non-reducing Glc H-1; 5.23 (d) α-reducing H-1; 4.66 (d, 8.0 Hz) β-reducing H-1	unchanged	1; 1; 1	Flour amylolysis of damaged starch; principal LAB substrate	5.41 overlaps sucrose Glc H-1 (5.41) and maltotriose/dextrin non-reducing ends; resolve by 2D or by subtracting sucrose measured from its fructose H-3 (4.22 d); 4.66 attenuated by water presaturation
Glucose	5.23 (d, 3.7 Hz) α-H-1; 4.64 (d, 8.0 Hz) β-H-1	unchanged	1; 1	Maltose phosphorylase product; sucrose hydrolysis; consumed by yeast	α-H-1 overlaps maltose α-reducing H-1; combine 5.23 total with maltose from 5.41 and sucrose to obtain free glucose by difference
Fructose	4.11 (d) β-furanose H-3; 3.5–4.1 (m)	unchanged	–	Sucrose and fructan hydrolysis; consumed as acceptor (mannitol) or by yeast	Tautomeric mixture; quantify by 4.11 d (overlaps lactate CH quartet at pH 7.4; use low-pH protocol or ^{13}C at δ 99.0/102.5 anomeric)
Sucrose	5.41 (d, 3.9 Hz) Glc H-1; 4.22 (d, 8.7 Hz) Fru H-3; 4.05 (t) Fru H-4	unchanged	1; 1; 1	Flour (0.5–1.5%); hydrolyzed within hours by yeast invertase and LAB	4.22 d is the clean resonance
Raffinose / fructo-oligosaccharides	4.99 (d) Gal H-1; 5.43 (d) Glc H-1; 4.2–4.3 (d) fructosyl H-3	unchanged	–	Flour; hydrolyzed by yeast invertase/inulinase and LAB fructanase	Galactosyl H-1 at 4.99 is diagnostic of raffinose-family oligosaccharides; fructan fructosyl H-3 signals at 4.2–4.3 track fructan degradation
Alanine	1.48 (d, 7.2 Hz) CH_3; 3.78 (q)	1.52	3	Proteolysis; alanine is enriched in gliadins; yeast release	Clean doublet; index of proteolysis
Valine	0.99, 1.04 (d) CH_3; 2.27 (m); 3.61 (d)	~unchanged	3; 3	Proteolysis; consumed by yeast (fusel alcohol precursor)	0.99/1.04 doublets overlap leucine/isoleucine; fit as group
Leucine / isoleucine	0.95, 0.96 (d); 0.94 (t), 1.01 (d)	~unchanged	–	Proteolysis; Ehrlich precursors of 3-methyl-1-butanol and 2-methyl-1-butanol	Branched-chain group 0.9–1.05 ppm; quantify by deconvolution or 2D
Threonine	1.33 (d) CH_3; 3.58 (d); 4.25 (m)	1.33	3	Proteolysis	Overlaps lactate CH_3 at pH 7.4; use 4.25 m or low-pH protocol
Glutamate	2.05, 2.12 (m) β-CH_2; 2.34 (m) γ-CH_2; 3.75 (dd) α-CH	2.1–2.2; 2.5	–	Proteolysis and glutaminase deamidation of glutamine (gluten is ~35% glutamine)	pH-sensitive; glutamate/glutamine ratio is an index of LAB glutaminase activity
Glutamine	2.14 (m); 2.45 (m); 3.77 (t)	–	–	Proteolysis; consumed by LAB glutaminase	Overlaps succinate/glutamate; 2D recommended
Proline	2.0–2.35 (m); 3.33, 3.41 (m); 4.13 (dd)	–	–	Proteolysis (gluten is ~15% proline)	4.13 dd near lactate CH quartet

Compound	Resonance (pH 7.4), δ ppm, multiplicity, J	At pH 2.5–3.0	Protons	Origin in sourdough	Quantification notes
γ-Aminobutyrate (GABA)	1.90 (m) β-CH_2; 2.30 (t) α-CH_2; 3.01 (t) γ-CH_2	1.95; 2.5; 3.02	2; 2; 2	LAB glutamate decarboxylase; absent or trace in yeast bread	3.01 t is diagnostic; 1.90 m overlaps acetate at pH 7.4 (resolved at low pH)
Ornithine	1.72–1.95 (m); 3.05 (t) δ-CH_2; 3.77 (t) α-CH	3.05	2	LAB arginine deiminase pathway; crust 2-acetyl-1-pyrroline precursor; absent in yeast bread	3.05 t adjacent to GABA 3.01 t; resolvable at 500–600 MHz; authentication marker
Citrulline	1.55, 1.87 (m); 3.14 (t) δ-CH_2; 3.75 (t)	–	2	Arginine deiminase intermediate	3.14 t
Arginine	1.65, 1.72, 1.91 (m); 3.24 (t) δ-CH_2; 3.77 (t)	–	2	Proteolysis; consumed via ADI pathway	3.24 t; depletion parallels ornithine formation
Phenylalanine	7.33 (d), 7.38 (t), 7.43 (t) ring; 3.12, 3.28 (dd); 3.99 (dd)	~unchanged	5	Proteolysis; precursor of 2-phenylethanol (yeast) and phenyllactate (LAB)	Ring multiplet; phenyllactate ring 7.2–7.4 overlaps
Tyrosine	6.90 (d), 7.19 (d) ring	~unchanged	2; 2	Proteolysis	Clean AA′BB′ pattern
Tryptophan	7.20, 7.28, 7.32 (m); 7.54 (d); 7.73 (d)	–	–	Proteolysis; precursor of indole-3-lactate (LAB)	Low concentration
Histidine	7.05 (s), 7.8–8.0 (s) imidazole	7.3, 8.6	1; 1	Proteolysis	Strongly pH-dependent
Betaine (glycine betaine)	3.26 (s) $N(CH_3)_3$; 3.90 (s) CH_2	unchanged	9; 2	Wheat germ and bran; increases with extraction rate	Clean 9-proton singlet; bran-content and whole-grain marker
Choline	3.20 (s) $N(CH_3)_3$; 3.52 (m); 4.07 (m)	unchanged	9	Flour phospholipid hydrolysis	3.20 s adjacent to betaine 3.26 s
Phenyllactate	7.2–7.4 (m); 4.28 (dd); 2.90, 3.10 (dd)	–	–	LAB reduction of phenylpyruvate; antifungal	Low concentration; near LOQ at 400 MHz
Acetoin / 2,3-butanediol	1.37 (d), 2.22 (s), 4.42 (q); 1.14 (d), 3.66 (m)	–	–	LAB and yeast pyruvate metabolism; diacetyl (2.32 s) precursor	Low concentration
1,3-Propanediol	1.78 (quintet); 3.66 (t)	unchanged	2; 4	*Limosilactobacillus reuteri* glycerol reduction	Present only with glycerol-reducing LAB
Citrate	2.53, 2.66 (d, AB, 15 Hz)	2.8–3.0	2; 2	Flour; consumed by some LAB (citrate lyase)	AB pattern; pH-sensitive
Malate	2.37, 2.68 (dd); 4.30 (dd)	2.6–2.9; 4.5	–	Flour; consumed by malolactic LAB	Overlaps succinate at pH 7.4
Uridine / adenosine / inosine	5.90 (d), 7.87 (d); 6.09 (d), 8.24, 8.35 (s); 6.10, 8.23, 8.34	–	–	Yeast autolysis; nucleoside release	Aromatic-region markers of yeast lysis in prolonged fermentation
Trimethylsilylpropionate (TSP-d_4)	0.00 (s)	0.00	9	Internal standard	Reference and quantification standard

Three features of the spectrum carry most of the information about the fermentation. The first is the sugar region: the sucrose fructosyl H-3 doublet at δ 4.22 ppm disappears within the first hours of fermentation, the raffinose galactosyl H-1 doublet at δ 4.99 ppm and the fructan fructosyl H-3 signals at δ 4.2–4.3 ppm decline more slowly, and the maltose non-reducing H-1 doublet at δ 5.41 ppm rises during the first hours as the amylases outpace consumption and then falls as the LAB and any maltose-fermenting yeast consume it, so that the trajectory of the maltose signal reports on the balance of amylolysis and fermentation.[4,19] The second is the aliphatic acid region: the lactate doublet at δ 1.33 ppm and the acetate singlet at δ 1.92 ppm grow in a ratio that is the fermentation quotient, the ethanol triplet at δ 1.18 ppm grows in proportion to the yeast

activity plus the acceptor-free heterofermentation, and the mannitol multiplets between δ 3.67 and 3.87 ppm appear only when heterofermentative LAB have had fructose available. The third is the amino acid region: the alanine doublet at δ 1.48 ppm, the branched-chain methyl cluster at δ 0.9–1.05 ppm, and the aromatic ring signals grow with proteolysis, while the ornithine δ-CH_2 triplet at δ 3.05 ppm and the GABA γ-CH_2 triplet at δ 3.01 ppm appear as LAB-specific products. The relative development of these three regions distinguishes a Type I sourdough from a yeast dough at a glance and, with quantification, provides the process indices of Section 7.3.

7.3 Quantification and the Tracking of a Fermentation

Quantitative ^{1}H NMR rests on the proportionality between the integrated intensity of a resonance and the number of nuclei it represents, which holds when the spectrum is acquired with a relaxation delay long relative to the longest T_1 of interest, with a uniform excitation profile, and with the receiver operating in its linear range.[40] The concentration of an analyte X is then obtained from the integral of a resolved resonance and that of the internal standard according to

$$c_X = (I_X / N_X) \times (N_{IS} / I_{IS}) \times c_{IS} \tag{9}$$

where I is the integral, N the number of protons contributing to the integrated resonance, and c the molar concentration, and the content of the analyte in the original starter, dough, or crumb follows from the extraction ratio and the molar mass:

$$w_X\ (\mathrm{g\ kg^{-1}}) = c_X\ (\mathrm{mol\ L^{-1}}) \times M_X\ (\mathrm{g\ mol^{-1}}) \times V_{extract}\ (\mathrm{L}) / m_{sample}\ (\mathrm{kg}) \times f_{dilution} \tag{10}$$

with f_dilution accounting for the buffer addition. For the small molecules of aqueous extracts at 400–600 MHz the longitudinal relaxation times of the methyl and methine protons lie between about 1 and 4 s, and a relaxation delay of 25–30 s with a 90° pulse, or of 10 s with a 30° pulse, satisfies the five-T_1 criterion; 32–64 transients give a limit of quantification of the order of 20–50 µmol L^{-1} in the extract, which corresponds to roughly 0.01–0.02 g kg^{-1} of lactate in the dough, three orders of magnitude below the concentrations produced by a sourdough fermentation. Water suppression by presaturation or by a one-dimensional NOESY-presaturation sequence attenuates resonances within about 0.1 ppm of the water signal at δ 4.7–4.8 ppm, which affects the β-anomeric protons of glucose and maltose at δ 4.64–4.66 ppm and is the reason that the α-anomeric and non-reducing-end signals are preferred for sugar quantification. Precision for the major analytes (lactate, acetate, ethanol, maltose, alanine) is 1–3% relative standard deviation on replicate extractions; for mannitol, glycerol, and the sugar signals that require deconvolution of the congested δ 3.5–4.0 ppm region it is 5–10%, and for these analytes a ^{1}H–^{13}C HSQC or a ^{13}C spectrum of a concentrated extract provides better-resolved signals at the cost of acquisition time.

A fermentation is tracked by sampling the starter or dough at intervals—for a Type I refreshment cycle, at 0, 2, 4, 6, 8, 12, and 24 h is a reasonable schedule—and by expressing the results as concentrations per kilogram of dough, as concentrations per kilogram of flour (which is what the microbiological literature reports and which allows comparison across dough yields), and as the derived indices: the fermentation quotient of Equation 5, the mannitol-to-acetate molar ratio (which is unity when fructose is the sole acceptor and falls below unity when oxygen, citrate, or fructose limitation intervene), the sum of lactate and acetate in millimoles per kilogram (which, together with the buffering capacity of the flour, determines the pH and which tracks the titratable acidity closely enough to serve as a cross-check on it), the ethanol-to-lactate ratio (which indexes the relative contribution of yeast and LAB), and the ornithine-to-arginine ratio (which indexes the activity of the arginine deiminase pathway). The titratable acidity measured on the dough and the acid sum measured by NMR will not agree exactly, because the titration also consumes base in

neutralizing the phosphate, protein, and inositol phosphate buffers of the flour and because the NMR sum omits the minor acids; the difference between them is itself informative about the buffering capacity of the flour and should be reported rather than reconciled by adjustment. Typical values for a mature Type I wheat sourdough of dough yield 200 at 24 h are lactate 8–15 g kg^{-1} dough, acetate 2–4 g kg^{-1}, ethanol 4–10 g kg^{-1}, mannitol 1–5 g kg^{-1}, glycerol 0.5–1.5 g kg^{-1}, and a fermentation quotient of 3–6; a rye sourdough at the same dough yield gives higher lactate and acetate because of the greater maltose supply and buffering capacity of rye; and a Type II sourdough after 48 h at 35–40 °C gives lactate of 20–40 g kg^{-1} with a fermentation quotient above 8 and little ethanol.[1,2,21] These ranges, which are collated from the compositional data of the cited reviews, define what a laboratory should expect to see; a sample outside them is either an unusual process or an unusual extract, and the distinction should be pursued.

7.4 ^{13}C, Two-Dimensional, and Diffusion-Ordered NMR

Carbon-13 NMR of a concentrated extract complements the proton spectrum by dispersing the sugar and polyol resonances across 60 ppm, so that mannitol (δ 64.4, 70.3, and 72.1 ppm), glycerol (δ 63.5 and 73.1 ppm), the anomeric carbons of glucose (δ 93.0 α and 96.8 β), maltose (δ 100.5 non-reducing C-1), sucrose (δ 93.0 Glc C-1, 104.7 Fru C-2), and fructose (δ 99.0 and 102.5 for the β-pyranose and β-furanose anomers), and the carboxylate carbons of lactate (δ 183.4 ppm) and acetate (δ 182.0 ppm) are resolved without deconvolution. The low natural abundance and long relaxation times of ^{13}C make the experiment slow, and it is normally used to confirm assignments and to quantify the polyols in a subset of samples rather than as a routine method. Two-dimensional ^{1}H–^{1}H COSY and TOCSY spectra trace the spin systems of the amino acids and organic acids and are the means by which the overlapping multiplets of the δ 1.7–2.5 ppm region (acetate, GABA, glutamate, glutamine, proline, arginine, ornithine) are disentangled, and a ^{1}H–^{13}C HSQC spectrum, which resolves each protonated carbon at its own carbon shift, is the most reliable single experiment for the identification and semi-quantification of the sugars and polyols in the congested region.[17] The metabolomic study of lactic-fermented common-bean flour by Tatulli et al. illustrates the approach applied to a legume substrate, in which the depletion of raffinose-family oligosaccharides and the accumulation of lactate, acetate, and free amino acids were followed by ^{1}H NMR with two-dimensional confirmation.[17]

The exopolysaccharides and the arabinoxylans are characterized in the polymer fraction retained by ultrafiltration or recovered by ethanol precipitation. The dextrans of *Leuconostoc* and *Weissella* give a dominant anomeric proton near δ 4.97 ppm for the α-(1→6)-linked backbone glucose with a smaller anomeric signal near δ 5.32 ppm for α-(1→3)-linked branch residues, and anomeric carbons near δ 98.5 and 100.5 ppm respectively, so that the degree of branching is obtained from the anomeric integrals; the levans of *F. sanfranciscensis* and *Lactobacillus* species, which have no anomeric proton, are identified by the fructofuranosyl C-2 quaternary carbon near δ 104.5 ppm and the characteristic set of six fructosyl carbon resonances, and the two-dimensional assignments of Maina et al. for the *W. confusa* and *L. citreum* polymers provide the reference data.[29] A dextran is distinguished from the maltodextrins generated by amylolysis by its anomeric shift (δ 4.97 versus δ 5.40 ppm for the α-(1→4) linkage) and, more robustly, by diffusion.

Diffusion-ordered spectroscopy (DOSY) resolves the components of a mixture by their translational diffusion coefficients, which are related to hydrodynamic radius by the Stokes–Einstein relation,

$$D = k_B T / (6 \pi \eta r_H) \tag{11}$$

so that small metabolites, oligosaccharides, and polysaccharides of different size appear at different positions along the diffusion dimension while retaining their chemical-shift resolution. Weckx et al. applied ^{1}H DOSY at elevated temperature—which lowers the viscosity of the arabinoxylan solutions and sharpens the polymer resonances—to the water-extractable arabinoxylans of wheat flour before and after sourdough fermentation and showed that fermentation alters the molecular structure of the polymer population toward smaller, more mobile species, consistent with the action of the endogenous and microbial xylanases and with the release of the arabinoxylan-bound ferulic acid described in Section 4.5.[26] The same experiment separates dextran and levan from the dextrins and free sugars of a sourdough extract in a single measurement and offers a route to the molecular-size distribution of the exopolysaccharides that does not require size-exclusion chromatography.

7.5 ^{31}P NMR of Phytate and Its Degradation Products

Phosphorus-31 NMR is the definitive method for the speciation of the inositol phosphates, because the six phosphate groups of myo-inositol hexakisphosphate (IP_6) and the phosphates of its partially dephosphorylated products IP_5, IP_4, IP_3, and the lower esters give resolved resonances whose chemical shifts depend on the position of the phosphate on the inositol ring and on the state of protonation, whereas chromatographic and colorimetric methods either do not distinguish the lower esters or require their separation.[25,46] The measurement is made at a fixed high pH, usually 12–13, at which all the phosphate groups are fully deprotonated and the shifts are stable; at this pH the four chemically distinct phosphorus environments of IP_6 (the axial P-2, the equatorial P-1/P-3, P-4/P-6, and P-5) give four resonances in the intensity ratio 1:2:2:1 within a window of about 2 ppm, orthophosphate appears as a separate singlet, and the lower inositol phosphates appear as additional resonances that are assigned by comparison with authentic standards and by the pH-titration behavior of each signal. The quantification protocols established by Kemme et al. for feed ingredients and applied by Reale et al. to dough share their essential features: extraction of the sample with dilute hydrochloric acid to dissociate the phytate from its calcium, magnesium, and potassium salts; adjustment to high pH with sodium hydroxide in the presence of a chelator such as EDTA, which sequesters the divalent and paramagnetic cations that would otherwise broaden the lines; acquisition with inverse-gated ^{1}H decoupling to suppress the nuclear Overhauser effect and with a relaxation delay long enough for the ^{31}P T_1 of several seconds; and quantification against a phosphorus standard whose resonance lies outside the inositol phosphate window.[25,46] Reale et al. applied ^{31}P NMR to wholemeal wheat dough fermented with *Lactobacillus plantarum*, *Levilactobacillus brevis*, *Latilactobacillus curvatus*, and *S. cerevisiae* strains isolated from southern Italian sourdoughs, assaying the phytase activity associated with each organism through the loss of the IP_6 resonances and the appearance of the lower inositol phosphates and orthophosphate during the fermentation; the same group subsequently showed that the sourdough LAB contribute little phytase of their own and that their principal role is to establish the pH at which the cereal phytase is most active (Section 4.5).[24,25] For a laboratory this is a two-hour experiment on a 400 MHz instrument with a broadband probe, and it answers, without calibration curves, the question of how much of the phytate of a whole-grain flour survives a given sourdough process and in what form; the nutritional literature holds that IP_3 through IP_6 inhibit mineral absorption while the lower esters do not, so that the speciation, and not merely the loss of IP_6, is the relevant quantity.[23,24] The same experiment on a chloroform–methanol extract with a cesium-EDTA aqueous phase resolves the phospholipids of the flour (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and the lysophosphatidylcholine that dominates the starch-bound lipid) and can follow their hydrolysis by the flour and microbial phospholipases during fermentation.

7.6 Organic Extracts and the Lipid Fraction

A chloroform–methanol extract of flour, dough, or crumb, taken up in $CDCl_3$ for ^1H and ^{13}C NMR, describes the lipid fraction in terms familiar from the edible-oil analysis practiced in the author's laboratory. The triacylglycerol glycerol backbone gives its doublets of doublets at δ 4.14 and 4.29 ppm and its multiplet at δ 5.26 ppm; the olefinic protons at δ 5.35 ppm, the bis-allylic methylene of linoleate at δ 2.77 ppm (wheat flour lipid is roughly 55–60% linoleate), the allylic methylenes at δ 2.02 ppm, and the α-methylene at δ 2.30 ppm give the unsaturation profile; and the ^{13}C carbonyl region distinguishes the esterified acyl chains (δ 172.8–173.3 ppm, with the sn-2 and sn-1,3 positions resolved) from the free fatty acids (δ 178–180 ppm), so that the extent of lipolysis, which is substantial in sourdough because of the acid-tolerant lipases of the flour and of some LAB and yeasts, is obtained from the ratio of free to esterified carbonyl. The galactolipids that constitute a large fraction of wheat flour polar lipid (monogalactosyl- and digalactosyldiacylglycerol) are identified by their galactosyl anomeric protons near δ 4.2–4.9 ppm in $CDCl_3$–CD_3OD. Lipid oxidation, which is promoted by the lipoxygenase of the flour during mixing and retarded by the low pH and by the antioxidant phenolics released during sourdough fermentation, is followed through the conjugated-diene olefinic multiplets between δ 5.5 and 6.6 ppm and the hydroperoxide proton near δ 8.2 ppm, and the hydroxylated and epoxidized fatty acids produced from linoleate by certain sourdough lactobacilli and identified as antifungal metabolites (Section 4.7) are detectable in a concentrated extract through their carbinol protons near δ 3.6 ppm and their oxirane protons near δ 2.9 ppm.[2,21] The lipid fraction is not a primary analytical target in most sourdough work, but it bears on flavor (the lipoxygenase-derived aldehydes), on dough rheology (the surface-active polar lipids that stabilize gas cells), and on staling (amylose–lipid complexation), and the extract is inexpensive to obtain from a sample that has already been taken for the aqueous work.

7.7 Chemometric Treatment and the Authentication of Sourdough Bread

The ^1H NMR spectrum of an extract is a fingerprint of some hundreds of resolved intensities, and multivariate analysis of a set of such fingerprints is the natural way to classify samples and to search for the resonances that discriminate between classes. The practice is well established for flours and breads: Brescia et al. combined HR-MAS ^1H NMR of intact samples with stable-isotope ratio measurements to characterize flours and breads from southern Italy, and Lamanna et al. used ^1H NMR profiling of durum wheat extracts, with multivariate analysis of the metabolite fingerprint, to relate durum samples to their geographical origin.[15,45] For sourdough the same tools can address the classification of starters by type and by dominant organism, the comparison of flours by their fermentation behavior, the monitoring of process drift in a bakery, and the authentication of the finished product. The pitfalls are those of any chemometric study on a small, heterogeneous data set, and they deserve plain statement because the sourdough literature contains examples of each. Spectra must be aligned, which requires the pH control of Section 7.1; they must be normalized to a quantity that does not itself carry the class information (the internal standard and the sample dry matter, rather than total spectral area, which in a sourdough is dominated by the sugars and acids that define the classes); they should be scaled so that the abundant sugars do not dominate the model; and the model must be validated on samples that were not used to build it, with permutation testing to establish that the discrimination exceeds chance. A principal-component model of twenty samples from two bakeries will separate the bakeries, and it will do so as readily on the basis of the flours, the dough yields, and the extraction batches as on the basis of the sourdough microbiota; the discriminating loadings must be read back into the chemistry of Sections 4 and 5 before any claim is made about what the separation means. Targeted quantification of the metabolites of Table 6, followed by conventional statistics on the concentrations, is more transparent than untargeted modeling of the binned spectrum and is recommended

as the first analysis of any sourdough data set; the untargeted model is then a check for discriminating features that the targeted list missed.

The authentication problem is specific and commercially important. A bread labeled as sourdough may have been leavened by a Type I starter without added baker's yeast; leavened by baker's yeast with a Type I, II, or III sourdough added as an ingredient for flavor and shelf life; or leavened by baker's yeast and acidified with purchased lactic acid, acetic acid, or vinegar, with or without flavoring. Consumer expectation and, in some jurisdictions, labeling guidance attach to the first of these, and the analytical laboratory is asked to distinguish among them from the finished loaf. Pontonio et al. approached the problem microbiologically, by quantitative PCR for the DNA of the sourdough LAB in bread, and their finding that pH and the contents of lactic and acetic acid in the breads were only weakly correlated with the bacterial load is the essential caution for the chemist: acid content alone does not authenticate a sourdough, because acid can be added.[47] What cannot readily be added, or is not added because it is not a recognized ingredient, is the set of co-metabolites that a living lactic fermentation produces in a dough alongside the acids, and it is the pattern of these co-metabolites, measured together with the acids in the ^{1}H NMR spectrum of a crumb extract, that constitutes a defensible chemical criterion. Table 7 sets out the criterion. A genuine Type I sourdough bread shows lactate and acetate at a fermentation quotient in the range of the process, mannitol whenever heterofermentative LAB were present, ornithine and GABA, an amino acid profile elevated several-fold above that of the flour, depletion of maltose relative to a yeast bread from the same flour, and low succinate; a yeast bread acidified with added acids shows lactate and acetate, often at an unnatural fermentation quotient (pure lactic acid gives a quotient that tends to infinity; vinegar gives a quotient near zero), no mannitol, no ornithine, negligible GABA, a yeast-bread amino acid profile, residual maltose, and the succinate of yeast metabolism at the level of a straight dough; and a yeast bread containing a sourdough ingredient shows the LAB co-metabolites at concentrations consistent with the dosage of the ingredient (5–20% of flour), together with the succinate and residual maltose of the yeast leavening. The criterion is stated as a pattern rather than a threshold because the concentrations depend on flour, process, and dosage, and it should be applied with reference samples from the same flour whenever possible. Two supplementary measurements strengthen it. The enantiomeric composition of the lactate, which NMR cannot provide but which an enzymatic D-/L-lactate assay provides in an hour, distinguishes the DL-lactate of sourdough lactobacilli from the essentially pure L-lactate of commercial fermentation-grade lactic acid; and the ^{13}C isotope ratio or the site-specific ^{2}H distribution of the acetic acid, obtainable by isotope-ratio mass spectrometry or by SNIF-NMR, distinguishes a petrochemical or a C_4-plant acetic acid from one fermented from the C_3 sugars of wheat. Neither is necessary in the clear cases; both are advisable when the NMR pattern is intermediate and the finding will be contested.

Table 7. A co-metabolite criterion for the classification of a bread labeled "sourdough," based on the ^{1}H NMR spectrum of an ultrafiltered aqueous crumb extract, with supplementary measurements. Concentrations refer to crumb. The criterion is a pattern; no single row is decisive.

Observable (Table 6 resonance)	Type I sourdough bread (starter-leavened)	Yeast bread with sourdough ingredient (Type II/III, 5–20% of flour)	Yeast bread with added lactic/acetic acid or vinegar	Yeast bread (control)
Lactate (1.33 d)	5–15 g kg^{-1}	1–6 g kg^{-1}, scaling with dosage	Any value; set by addition	< 0.5 g kg^{-1}
Acetate (1.92 s)	1–4 g kg^{-1}; FQ 2–6	0.3–2 g kg^{-1}; FQ of the ingredient (often > 5)	FQ extreme: $\gg$ 10 (lactic acid only) or $\ll$ 1 (vinegar only) unless both added	Trace

Observable (Table 6 resonance)	Type I sourdough bread (starter-leavened)	Yeast bread with sourdough ingredient (Type II/III, 5–20% of flour)	Yeast bread with added lactic/acetic acid or vinegar	Yeast bread (control)
Mannitol (3.87 dd; ^{13}C 64.4)	Present (1–8 g kg^{-1}) when heterofermentative LAB and fructose present; may be low in homofermentative Type I doughs	Present in proportion to dosage if ingredient was heterofermentative	Absent	Absent
Ornithine (3.05 t)	Present, 0.1–1 g kg^{-1}	Present at low level	Absent	Absent
GABA (3.01 t)	Present	Present at low level	Absent or trace	Trace
Free amino acids (alanine 1.48 d; branched-chain 0.9–1.05)	Elevated several-fold over flour; depleted only if yeast co-fermentation prolonged	Modestly elevated	Yeast-bread level (low; consumed by yeast)	Low
Maltose (5.41 d)	Depleted relative to control	Intermediate	At yeast-bread level (residual maltose present)	Residual maltose present
Succinate (2.41 s)	Low (0.2–1 g kg^{-1}) unless baker's yeast was also added	Yeast-bread level	Yeast-bread level (0.5–2 g kg^{-1})	0.5–2 g kg^{-1}
Glycerol (3.56 dd)	Present	Present	Present	Present
Ethanol (1.18 t)	Trace (lost on baking)	Trace	Trace	Trace
Phytate (^{31}P) in whole-grain products	Substantially hydrolyzed; lower inositol phosphates present	Partly hydrolyzed	Largely retained	Largely retained
D-lactate fraction (enzymatic assay; not NMR)	30–60% (species-dependent)	Reflects ingredient organisms	~0% if commercial L-lactic acid; ~50% if racemic synthetic	Not applicable
Acetic acid $\delta^{13}C$ / SNIF-NMR (external)	C_3 fermentation signature	C_3 fermentation signature	Depends on source of acid; petrochemical or C_4 signature possible	Not applicable

Two limitations of the criterion should be recorded. A homofermentative or facultatively heterofermentative Type I starter (for example one dominated by *L. plantarum* or *Companilactobacillus paralimentarius*) in a flour poor in fructose will produce a genuine sourdough with little mannitol and little acetate, so that the absence of mannitol is not evidence against a lactic fermentation, only the absence of a heterofermentative one; ornithine and GABA, which are formed by a wider range of LAB, and the amino acid and maltose patterns are then the decisive observables. And a sourdough ingredient dosed generously into a yeast dough can reproduce the co-metabolite pattern of a starter-leavened bread in every respect except the succinate and the residual maltose, which are the marks of the yeast leavening; the distinction between "made with sourdough" and "leavened with sourdough" is therefore the hardest case, and the laboratory should say so in its report rather than force a classification.

8. High-Resolution Magic-Angle Spinning and Solid-State NMR of Flour, Dough, and Bread

8.1 HR-MAS ^1H NMR of Intact Samples

High-resolution magic-angle spinning NMR obtains liquid-like ^1H spectra from semi-solid samples by spinning a few tens of milligrams of the intact material, swollen with D_2O, at 4–6 kHz about an axis inclined at 54.7° to the magnetic field, which averages the residual dipolar couplings and the magnetic-susceptibility

broadening that would otherwise obscure the spectrum of a heterogeneous, water-rich matrix. The mobile constituents—free sugars, organic acids, amino acids, polyols, the mobile acyl chains of the lipids, and the mobile segments of solvated polysaccharides—are observed with line widths of a few hertz, while the rigid biopolymers (crystalline starch, the gluten network, and the cell-wall material) contribute only a broad underlying hump that is removed by a Carr–Purcell–Meiboom–Gill (CPMG) T_2 filter. Brescia et al. applied ^{1}H HR-MAS NMR, together with stable-isotope ratio measurements, to flours and breads produced in southern Italy and obtained from the intact samples the signals of the lipid unsaturation, the sugars, and the amino acids that, treated by multivariate statistics, characterized the samples without any extraction step.[15] The attractions for sourdough work are the absence of extraction—and therefore of the extraction-dependent selectivity discussed in Section 7.1—and the speed, since a starter or dough sample can be packed into a rotor and measured within minutes of sampling. The limitations are equally real. The method is at best semi-quantitative, because the rotor holds an ill-defined mass of a heterogeneous material, because the signal of any analyte that is partly immobilized by binding to the biopolymers is attenuated by the T_2 filter, and because the centrifugal field at the rotor wall can separate the liquid phase of a dough from its solid phase during the measurement. HR-MAS is accordingly best used for rapid fingerprinting and classification and as a complement to the extract-based quantification of Section 7, rather than as a replacement for it.

8.2 ^{13}C CP/MAS NMR of Starch: Gelatinization, Retrogradation, and the Crust

Cross-polarization magic-angle spinning ^{13}C NMR of solid starch reports on the molecular order of the polymer through the fine structure of the C-1 resonance near δ 100 ppm and through the position and shape of the C-4 resonance near δ 82 ppm and the C-6 resonance near δ 62 ppm. Gidley and Bociek established the interpretation: in the A-type crystalline polymorph of cereal starches the C-1 resonance is a triplet spanning roughly δ 99–102 ppm, reflecting three crystallographically distinct glucose environments in the unit cell; in the B-type polymorph of tuber starches and of retrograded amylopectin it is a doublet; in amorphous starch it is a single broad resonance centered near δ 102–103 ppm with a shoulder toward higher shift; and the C-4 resonance shifts from about δ 82 ppm in the amorphous material to a resolved signal near δ 80 ppm in the double-helical material, so that the fraction of double helices can be estimated independently of, and is generally larger than, the crystallinity measured by X-ray diffraction, because double helices exist in the granule that are not packed into crystalline registers.[48] A fourth pattern, the V-type single helix of the amylose–lipid complex, gives a C-1 resonance near δ 103–104 ppm and a distinct C-4 signal, and it is the polymorph that forms during baking as the leached amylose complexes with the flour lipids.

Morgan et al. applied these assignments to bread and observed, in the spectra of dough, fresh bread, and stored bread, the sequence that defines the starch chemistry of Section 5: the A-type triplet of the flour starch is preserved in the dough, is lost on baking as the granules gelatinize and the starch becomes amorphous with a superimposed V-type contribution from the amylose–lipid complex, and is replaced on storage by the growing signature of the B-type double helices of retrograded amylopectin.[49] Baik et al. followed the aging of starch in white bread by ^{13}C CP/MAS NMR over storage and related the growth of the ordered-starch features to the firming of the crumb and to its moisture, and Primo-Martín et al., combining ^{13}C CP/MAS NMR with X-ray diffraction and differential scanning calorimetry across flour, crumb, and crust, showed that the C-1 triplet of the flour starch is lost in the crumb but that the crust, which dehydrates before its starch can fully gelatinize, retains a substantial fraction of the native crystallinity—of the order of 30–40% relative crystallinity in fresh crust by both diffraction and calorimetry—and that this crust crystallinity increases further on storage by amylopectin retrogradation.[41,50] The measurements are made on freeze-dried or native-moisture samples packed into 4 mm rotors, with contact times of 1–2 ms and recycle delays of a few seconds, and they require a solid-state probe on a wide-bore or standard-bore magnet; because cross-

polarization is not quantitative across environments of differing rigidity, the comparison of double-helix content between samples of the same type is more secure than an absolute determination, and a direct-polarization spectrum with a long recycle delay is the reference when absolute values are needed. Nowacka-Perrin et al. have shown that the combination of cross-polarization, which selects the rigid carbons, with INEPT polarization transfer, which selects the mobile carbons, and direct polarization, which sees both, followed in situ as a sample is heated and cooled, partitions the starch carbons into rigid and mobile fractions and follows gelatinization and retrogradation in real time in a food matrix.[51]

For sourdough these methods answer questions that no extract can: whether the acidification, the dextrins generated by the acid-activated amylases, the solubilized arabinoxylans, and the exopolysaccharides of a given process actually reduce the rate and extent of amylopectin recrystallization in the crumb, as the anti-staling literature of Section 5.4 asserts, and whether the crust of a sourdough loaf, which is formed at a lower pH and with a different sugar and amino acid inventory, differs in its retained crystallinity from that of a yeast loaf. The experiment is simple in design—matched sourdough and yeast loaves from the same flour, crumb and crust sampled at intervals over a week of storage, freeze-dried and measured—and the double-helix content from the C-1 and C-4 line shapes, compared with firmness and with the low-field relaxometry of Section 9, would give a direct test of the mechanism.

8.3 Solid-State NMR of Gluten and of Water–Biopolymer Interactions

The gluten proteins have been studied by ^{13}C and ^{1}H solid-state NMR principally as a function of hydration, because the transition of gluten from a glassy, rigid solid at low water content to the mobile, viscoelastic network of a dough is a plasticization by water that the relaxation and line-narrowing behavior of the protein report directly. Belton's loop-and-train model was constructed on solid-state NMR and infrared evidence that hydration mobilizes the glutamine-rich repetitive domains of the high-molecular-weight glutenin subunits selectively, and Calucci et al. characterized the hydration dependence of gluten dynamics through ^{13}C CP/MAS spectra, in which the resonances of the mobilized side chains narrow and their cross-polarization efficiency falls as water is added, and through ^{1}H free-induction decays and relaxation times, which resolve a rigid and a mobile proton fraction whose proportions change with water content.[35,42] Kim-Shin et al. used ^{17}O NMR, which observes the water molecule itself rather than the exchange-averaged proton pool, to follow water mobility during bread staling and reported a decrease in water mobility over storage that was modest in relation to the change in firmness, an early indication that the immobilization of water accompanies staling but does not by itself account for it.[52] These approaches have not been applied specifically to sourdough gluten, and the question they could address is well defined: the acid- and reductant-modified gluten of a sourdough is more extensible and holds more water than the gluten of a yeast dough, and the proton and ^{13}C dynamics of freeze-dried gluten isolated from the two doughs, measured as a function of rehydration, would show whether the difference resides in the mobility of the protein, in the partitioning of the water, or in both.

9. Low-Field Time-Domain NMR Relaxometry of Dough and Bread

9.1 Principles and Measurables

Time-domain NMR at low field measures the decay of the proton magnetization of a sample of several grams without resolving chemical shifts, and it derives from that decay the numbers and relaxation times of the proton populations present. Two experiments carry most of the information. The free-induction decay (FID) recorded immediately after a single pulse, with a dead time of a few microseconds, contains the

rapidly decaying signal of the protons of the rigid biopolymers—crystalline and glassy starch, the immobile fraction of the gluten, and the cell-wall material—whose transverse relaxation time T_2 is 10–20 µs and whose line shape is Gaussian or Abragam-like, together with the slower-decaying signal of more mobile protons. The Carr–Purcell–Meiboom–Gill (CPMG) echo train, which refocuses the field inhomogeneity, records the decay of the mobile protons over a range from a few hundred microseconds to hundreds of milliseconds, and its analysis by discrete multi-exponential fitting or by an inverse Laplace transform yields a distribution of T_2 values whose peaks are assigned to protons of differing mobility and environment. The longitudinal relaxation time T_1 is measured by inversion or saturation recovery and, in combination with T_2 in a two-dimensional T_1–T_2 correlation experiment, resolves populations that overlap in one dimension. In a water-rich, heterogeneous biopolymer system such as dough, the T_2 of the water protons is shortened by chemical exchange with the hydroxyl and amide protons of the biopolymers and by the restriction of water motion at the biopolymer surfaces, so that a measured T_2 reports on the intimacy of the contact between the water and the solid rather than on the water alone, and the assignment of a population to "water in the gluten network" or "water associated with starch" is an interpretation supported by model systems rather than a direct observation.[6] Instruments operating at 0.5–1.5 T (20–60 MHz) with 10–25 mm sample tubes, temperature control from 0 to 100 °C, and dead times of 5–10 µs are standard; the measurement takes a few minutes, is non-destructive, and can be repeated on the same sample through a fermentation, a heating ramp, or a week of storage.

9.2 The Proton Populations of Dough and Bread

Ruan et al. characterized the water in dough by relaxation measurements and resolved several populations of differing mobility whose proportions depend on water content, and Doona and Baik used FID and CPMG measurements on model doughs of starch, gluten, and water to relate the populations to the components.[53,54] The most systematic assignment is that of Bosmans et al., who measured flour, isolated starch, isolated gluten, and their mixtures at controlled hydration and then dough and bread, and who assigned the FID and CPMG populations of dough and bread by reference to the model systems.[7] Table 8 summarizes the population structure that emerges from that work and from the other cited studies, with the relaxation-time ranges expressed as ranges rather than as the specific values of any one study, since the values depend on field, temperature, moisture, and the fitting procedure. In dough, the FID contains a rigid population with T_2 of 10–20 µs, assigned to the non-exchanging CH protons of crystalline starch and rigid gluten, and a second, less rigid FID population, assigned to CH protons of amorphous starch and gluten that are plasticized by water; the CPMG decay contains two or three populations with T_2 from a few hundred microseconds to a few tens of milliseconds, assigned respectively to exchangeable biopolymer protons in fast exchange with water in intimate contact with the starch and gluten, to water within the gluten network and inside the granules, and to the most mobile extragranular water and any mobile lipid. In bread, gelatinization eliminates most of the rigid crystalline-starch population, redistributes water into the swollen starch, and produces a characteristic shift of the CPMG populations toward shorter T_2 as the water becomes more intimately associated with the gelatinized starch and the coagulated gluten.

Table 8. Proton populations resolved by low-field time-domain NMR in wheat dough and bread crumb, with the assignments established from starch, gluten, and flour model systems. Relaxation-time ranges are indicative at 20–25 °C and 20–60 MHz; absolute values depend on moisture, temperature, and the fitting model.

Experiment	Population	Approximate T_2	Assignment (dough)	Assignment and behavior (bread crumb)	Sensitivity to sourdough variables
FID	A (rigid)	10–20 µs (Gaussian/Abragam)	Non-exchanging CH protons of crystalline starch and glassy/rigid gluten	Reduced after gelatinization; increases during staling as amylopectin recrystallizes and gluten stiffens	Rate of increase during storage indexes staling; expected to be slowed by dextrins, exopolysaccharides, solubilized arabinoxylans
FID	B (semi-rigid)	50–200 µs	CH protons of amorphous starch and hydrated gluten, plasticized by water	Present; sensitive to moisture and to plasticization	Sensitive to acid-modified gluten and to water content
CPMG	C	0.3–3 ms	Exchangeable OH/NH protons of starch and gluten in fast exchange with tightly associated water	Increases in proportion after gelatinization as water enters the swollen granules and the coagulated protein	Sensitive to pentosans and exopolysaccharides that bind water; to pH through exchange rates
CPMG	D	3–20 ms	Water within the gluten network and inside the starch granules; less mobile water phase	Dominant water population of fresh crumb; T_2 decreases and proportion shifts during staling	Primary staling indicator in relaxometry; sourdough effects expected here
CPMG	E	20–200 ms	Most mobile extragranular water; mobile lipid; free water in high-hydration doughs	Small in crumb; larger in high-moisture and high-hydration formulations	Sensitive to dough yield and to added water-binding polymers
T_1	Single or two components	50–300 ms	Average over exchanging populations	Decreases with staling in some studies	Complementary; T_1–T_2 maps resolve overlapping populations

9.3 Mixing, Fermentation, and Proofing

During mixing the rigid FID population decreases and the mobile CPMG populations grow as the flour hydrates and the gluten is developed, and the T_2 of the water populations lengthens as water is redistributed from the surface of the flour particles into the gluten and the damaged starch; the changes are complete within the first minutes of mixing and are a convenient index of hydration and development.[53,54] Through fermentation and proofing the population structure changes more slowly, in response to the softening of the gluten, the generation of soluble metabolites (each gram of lactate, acetate, ethanol, mannitol, and free sugar adds to the mobile proton pool and, through its osmotic effect, changes the activity of the water), the solubilization of arabinoxylans, and the production of exopolysaccharides. The effects of these sourdough-specific variables on the relaxometry of dough have not been systematically reported, and Table 8 records expectations rather than findings; the one directly relevant model study is that of Tang et al., who used low-field NMR to show that a high-molecular-weight dextran produced by *W. confusa* altered the water distribution of a wheat starch gel and reduced its syneresis on freeze–thaw cycling, which is the mechanism by which an exopolysaccharide-producing sourdough is expected to improve the freeze–thaw stability and retard the staling of bread.[55] A study in which a Type I sourdough and a matched yeast dough are followed by FID and CPMG at intervals through proofing, with the metabolite inventory of Section 7 measured on parallel extracts, would establish whether the relaxometric signature of a sourdough proof is dominated by the acid-modified gluten, by the soluble metabolites, or by the polysaccharides, and it is proposed in Section 11.

9.4 Baking, Cooling, and Staling

Temperature-resolved relaxometry follows gelatinization directly. Rondeau-Mouro et al. measured the FID and CPMG populations of wheat starch model systems and of flour dough as a function of temperature and identified the transitions—the onset of granule swelling, the loss of the rigid crystalline population, the increase in the population of water in intimate contact with starch, and the changes in the exchange behavior—that accompany gelatinization under the limited-water conditions of a dough.[56] Engelsen et al. carried out "NMR-baking," in which the dough was baked inside the temperature-controlled probe of a low-field instrument and the relaxation data recorded throughout, and then used multivariate regression to predict the instrumental texture parameters of the resulting bread from the relaxation curves, demonstrating that the relaxometric record of the baking process carries the information that determines crumb texture.[57] Wagner et al. and Bajd and Serša have made the equivalent measurements by imaging (Section 10).

Staling has been the principal application. Curti et al. followed the crumb of wheat bread through storage by ^{1}H FID and CPMG relaxometry and observed the increase of the rigid population and the shortening of the T_2 of the water populations that accompany firming, and later evaluated whether two-dimensional T_1–T_2 relaxometry adds information to the one-dimensional analysis, concluding that the correlation maps resolve populations that overlap in one dimension at the cost of acquisition time.[43,58] Bosmans et al. combined relaxometry with calorimetry, texture analysis, and the model-system assignments of Table 8 to argue that crumb firming has two contributions: the recrystallization of amylopectin, which is what calorimetry measures, and the migration of water from the gluten to the crystallizing starch, which stiffens the protein network and which relaxometry detects as a change in the population structure that continues after the calorimetric endotherm has plateaued.[39] The interpretation matters for sourdough because the anti-staling mechanisms attributed to it act on different populations: dextrins and exopolysaccharides act on the amylopectin recrystallization, while the acid-modified, more hydrated gluten and the solubilized arabinoxylans act on the water partitioning, and relaxometry can separate the two where firmness measurements cannot. A protocol of matched loaves sampled daily for a week, each measurement taking five minutes, is well within the capacity of a contract laboratory and is the most efficient way to substantiate or refute a shelf-life claim for a sourdough process.

9.5 Benchtop High-Resolution NMR at 60–100 MHz

Between the relaxometers of Sections 9.1–9.4 and the high-field spectrometers of Section 7 stand the benchtop high-resolution instruments, with permanent magnets of 1.4–2.4 T and proton frequencies of 60–100 MHz, which resolve chemical shifts at reduced dispersion and sensitivity and which the author's laboratory operates alongside its 300 MHz instrumentation.[44] In the spectrum of a sourdough extract at 60 MHz the lactate methyl doublet (J = 6.9 Hz, spanning 0.115 ppm), the acetate singlet, the ethanol triplet, the succinate singlet, and the anomeric signals of maltose, glucose, and sucrose are resolved and quantifiable; the mannitol, glycerol, and sugar ring-proton region between δ 3.4 and 4.0 ppm is a single envelope that can be treated only by deconvolution or by chemometric calibration against high-field values; the ornithine and GABA triplets, 0.04 ppm apart, are not resolved from each other at 60 MHz and are marginally resolved at 80–100 MHz; and the aromatic region is usable for phenylalanine and tyrosine in concentrated extracts. With a freeze-dried extract reconstituted at 100–200 mg mL^{-1} and an acquisition of 15–30 min, limits of quantification of 0.5–1 mmol L^{-1} in the tube—of the order of 0.05–0.2 g kg^{-1} of lactate or acetate in the dough—are achieved, which is sufficient for the process indices of Section 7.3. The instrument is therefore adequate for the routine at-line monitoring of a starter or a Type II fermentation (lactate, acetate, fermentation quotient, ethanol, residual maltose) and for the quality control of sourdough ingredients, and it

is inadequate for the co-metabolite authentication of Section 7.7, which depends on ornithine, GABA, and mannitol and which should be referred to the high-field instrument.

10. Magnetic Resonance Imaging of Proofing, Baking, and Storage

Magnetic resonance imaging adds spatial resolution to the relaxometric measurements and is the only NMR method that reports on the structure of dough and bread at the length scale of the gas cell. Proton-density and T_2-weighted images of a proofing dough show the gas cells as signal voids in the signal-bearing dough matrix, and image analysis of a time series gives the number, size distribution, and growth rate of the cells and the porosity of the dough. van Duynhoven et al. established the quantitative approach, imaging dough during proofing and extracting the gas cell size distribution and its evolution by automated image analysis, and Rouillé et al. used the same measurement to show how the minor components of flour—the polar lipids and the water-soluble pentosans—affect bubble growth and coalescence during proofing, which is a direct observation of the mechanism by which these components determine crumb structure.[36,37] Bajd and Serša imaged dough continuously through fermentation and then through baking by magnetic resonance microscopy in a heated sample chamber, following the expansion of the gas cells, the loss of signal from the crust as it dehydrates, and the setting of the crumb, and Wagner et al. built a dedicated baking device for use inside a magnet, analyzed the evolution of the signal through baking, and related the local expansion and the loss of signal to the temperature and water-content histories of crumb and crust.[8,59] For storage, Lodi et al. imaged the water distribution in bread over days and characterized the migration of water from crumb toward crust and the changes in local signal intensity and relaxation that accompany staling, which locates in space the water redistribution that relaxometry measures in bulk.[60]

No magnetic resonance imaging study specific to sourdough has been located in the course of preparing this paper, and the section is therefore a statement of transferable methodology rather than of established sourdough results. The questions that the method could settle are nevertheless clear and are of practical importance to the artisan and industrial sourdough baker: whether the larger and more irregular gas cells of a sourdough crumb (Section 5.2) arise from a different rate of coalescence during proofing, as the acid-softened gluten would predict, or from a different distribution of cell nuclei at the end of mixing; whether the exopolysaccharides and solubilized arabinoxylans of a sourdough stabilize the gas cell walls in the way that Rouillé et al. observed for the native pentosans; and whether the slower crumb-to-crust water migration that the anti-staling literature attributes to sourdough is real. Imaging at low field on a benchtop or compact system, which resolves the gas cells of a proofing dough at a resolution of a few hundred micrometers, is sufficient for the first two questions, while the third requires the quantitative relaxation mapping of a high-field system.

11. A Proposed NMR Analytical Program for Process NMR Associates

11.1 Design Principles

The program set out in this section is designed for a laboratory equipped with 300 MHz superconducting instrumentation for solution-state ^{1}H, ^{13}C, ^{31}P, two-dimensional, and diffusion-ordered spectroscopy and with 60 MHz benchtop instruments capable of both high-resolution spectroscopy and relaxation measurements, and it is intended to be executed on matched pairs of sourdough and baker's-yeast doughs prepared from the same flour so that every measured difference can be attributed to the fermentation rather than to the raw material. The principles are those of Sections 6–10: extract-based quantification of the metabolites and polymers is the foundation; relaxometry of the intact dough and crumb is the process and

shelf-life monitor; solid-state ^{13}C is the arbiter of starch order where a staling mechanism is at issue; and every measurement is made on a defined sampling schedule against a matched control. Figure 2 maps the sampling points of the process onto the NMR observables, and Table 9 sets out the schedule, the sample preparation, the experiments, and the quantities to be reported. The experimental data, calculations, chemometric results, and interpretations arising from the execution of this program are to be supplied by the author and will be incorporated in version 1.1 of this paper; the subsections reserved for them are identified in Section 11.4.

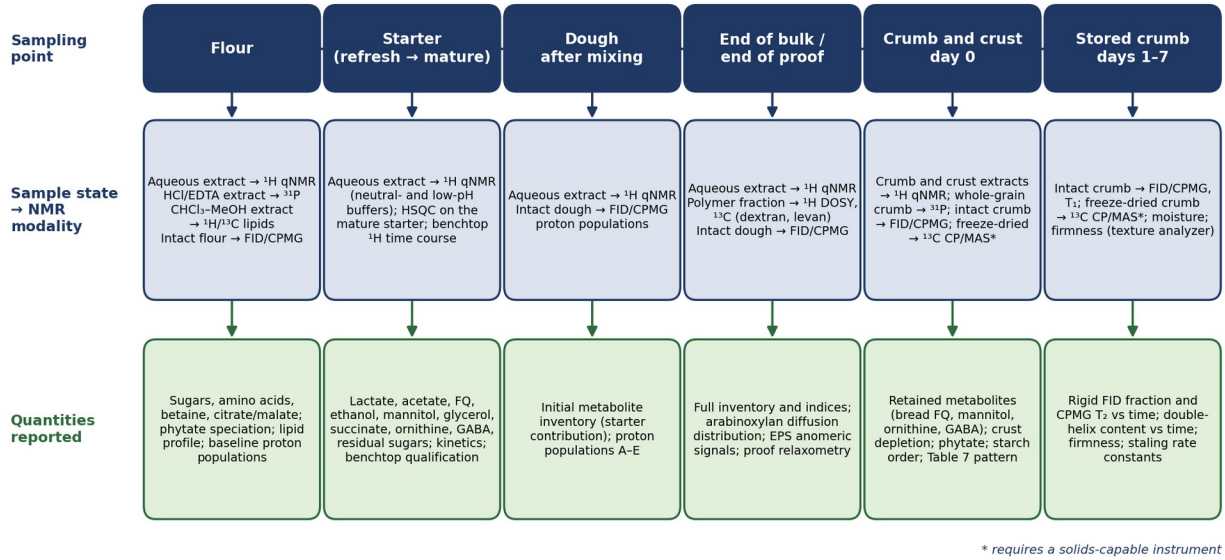

Figure 2. Mapping of the sourdough process onto the NMR observables and instruments of the proposed program. The upper row shows the sampling points from flour to stored loaf; the middle row shows the sample state and the NMR modality applied at each point; the lower row shows the quantities reported. Solid-state ^{13}C CP/MAS measurements require a solids-capable instrument and are shown for completeness. Schematic prepared for this white paper.

11.2 Sampling Schedule and Experiments

Table 9. Proposed sampling schedule, sample preparation, NMR experiments, and reported quantities for a matched sourdough versus baker's-yeast study on a single flour. Each row is executed for both doughs; the starter rows apply to the sourdough only. ^{1}H, ^{13}C, ^{31}P, 2D, and DOSY experiments are at 300 MHz; benchtop experiments are at 60 MHz.

Sampling point	Sample and preparation	NMR experiments	Quantities and indices reported	Purpose
Flour (as received)	Aqueous extract (1 g/10 mL), ultrafiltered; alkaline HCl/EDTA extract for ^{31}P; chloroform–methanol extract for lipids; 5 g intact for relaxometry	^{1}H qNMR; ^{31}P; $^{1}H/^{13}C$ of lipid extract; benchtop FID/CPMG	Sucrose, raffinose, fructans, maltose, glucose, fructose, free amino acids, betaine, citrate, malate; IP_6 and lower inositol phosphates; lipid unsaturation and free fatty acid fraction; baseline proton populations	Substrate inventory and baseline against which fermentation changes are expressed per kilogram of flour

Sampling point	Sample and preparation	NMR experiments	Quantities and indices reported	Purpose
Starter, freshly refreshed (t = 0) and mature (t = 8–24 h; sampled at 0, 2, 4, 8, 12, 24 h in the kinetic study)	2 g in 8 mL ice-cold water, ultrafiltered; pH and TTA recorded on the starter	^{1}H qNMR (neutral-buffer and low-pH protocols); HSQC on the mature sample; benchtop ^{1}H on all time points	Lactate, acetate, FQ, ethanol, mannitol, glycerol, succinate, residual maltose/glucose/fructose, alanine, branched-chain amino acids, ornithine, arginine, GABA, glutamate/glutamine ratio; comparison of benchtop with high-field values	Characterization of the starter and its fermentation kinetics; qualification of the benchtop method for at-line use
Dough after mixing (t = 0)	As for starter; 5 g intact in tube for relaxometry	^{1}H qNMR; benchtop FID/CPMG	Metabolite inventory at the start of bulk fermentation; proton populations A–E	Defines the initial state; the sourdough dough contains the starter contribution
End of bulk fermentation and end of proof	As above, plus retained non-ultrafiltered supernatant for polymer work	^{1}H qNMR; benchtop FID/CPMG on intact dough; ^{1}H DOSY and ^{13}C on the polymer fraction of the proofed dough	Full metabolite inventory and indices; relaxometric population changes through the proof; arabinoxylan diffusion-coefficient distribution; exopolysaccharide anomeric signals (α-(1→6) at δ 4.97 ppm; levan C-2 at δ 104.5 ppm)	Establishes what the fermentation has done to metabolites, water partitioning, and polymers before baking
Crumb and crust, day 0 (cooled 2 h)	Crumb: 5 g/20 mL extract, ultrafiltered; crust separately; ^{31}P extract of whole-grain crumb; 5 g intact crumb for relaxometry; freeze-dried crumb and crust for solid-state work	^{1}H qNMR of crumb and crust extracts; ^{31}P of whole-grain crumb; benchtop FID/CPMG; ^{13}C CP/MAS where available	Retained lactate, acetate (bread FQ), mannitol, ornithine, GABA, free amino acids, residual sugars, succinate; crust depletion of amino acids and sugars; residual phytate speciation; baseline crumb populations; starch double-helix content	Finished-product characterization; authentication reference pattern (Table 7); baseline for staling
Crumb, days 1, 3, 5, 7 (sealed storage at 22 °C)	5 g intact crumb for relaxometry; freeze-dried crumb for solid-state work; crumb moisture by loss on drying; firmness by texture analysis	Benchtop FID/CPMG (and T_1 where available); ^{13}C CP/MAS where available	Rigid FID fraction and CPMG population T_2 and amplitudes versus time; double-helix content versus time; firmness versus time; fitted staling rate constants	Direct test of the anti-staling effect of the sourdough process against the matched yeast control
Commercial "sourdough" breads (survey set)	Crumb extracts as for day 0 crumb	^{1}H qNMR; HSQC for mannitol; enzymatic D-/L-lactate on a subset	Table 7 co-metabolite pattern for each product; classification	Application of the authentication criterion to market samples; identification of products acidified without lactic fermentation

11.3 Reporting Conventions

Metabolite concentrations are to be reported both per kilogram of dough or crumb as analyzed and per kilogram of flour, the latter calculated from the dough yield and, for bread, from the bake loss, so that the results are comparable with the microbiological literature, which reports per flour, and with the food-composition literature, which reports per product. Each reported value is to carry the extraction ratio, the buffer protocol (neutral or low-pH), the internal standard, and the resonance integrated, because the tabulated shifts and overlaps of Table 6 make the last of these a material detail; the pH of each NMR sample after buffering is to be recorded. The relaxometric data are to be reported as the fitted amplitudes and relaxation times of each population with the fitting model stated (discrete exponential or inverse Laplace),

together with the crumb moisture at each time point, since a population change without the accompanying moisture is uninterpretable. Chemometric analyses of the extract spectra, should they be undertaken on the survey set, are to be reported with the number of samples, the preprocessing (alignment, normalization, scaling), the validation scheme, and the permutation-test result, in keeping with the cautions of Section 7.7, and the study inputs and outputs are to be archived as spreadsheets accompanying the report in accordance with the laboratory's standing practice for chemometric work.

11.4 Reserved Subsections for Experimental Results (Version 1.1)

The following subsections are reserved for the author's experimental data and interpretation and are intentionally empty in version 1.0: 11.4.1 Flour substrate inventory; 11.4.2 Starter kinetics by high-field and benchtop ^{1}H NMR; 11.4.3 Metabolite inventory of matched doughs at end of proof; 11.4.4 Polymer fraction: arabinoxylan DOSY and exopolysaccharide signals; 11.4.5 ^{31}P speciation of phytate in whole-grain crumb; 11.4.6 Relaxometric staling kinetics of matched loaves; 11.4.7 Solid-state ^{13}C starch order in crumb and crust; 11.4.8 Survey of commercial sourdough breads against the co-metabolite criterion.

12. Critical Assessment: What Is Established, What Is Plausible, and What NMR Cannot Resolve

The literature reviewed in this paper supports a set of established conclusions that a laboratory may state without qualification. The metabolic pathways of Section 4—maltose phosphorylase and the phosphoketolase pathway, the use of fructose and oxygen as electron acceptors with the formation of mannitol and acetate, the glutathione-reductase-mediated thiol exchange, the arginine deiminase pathway, and the yeast–bacterium partnership of *F. sanfranciscensis* with maltose-negative yeasts—are established by decades of biochemical and genetic work and are the basis on which the ^{1}H NMR spectrum of a sourdough extract is interpreted.[2–4] The transformations of starch on baking and staling, and the capacity of ^{13}C CP/MAS NMR to observe them, are established.[48–50] The population structure of dough and bread in low-field relaxometry and its response to gelatinization and staling are established, subject to the interpretive caveat that population assignments rest on model systems.[7,39] The degradation of phytate during sourdough fermentation and the capacity of ^{31}P NMR to speciate the products are established.[23,25] The general NMR-detectable differences between sourdough and yeast bread—acid inventory, mannitol, ornithine, amino acid enrichment, sugar depletion, succinate—follow from the established biochemistry and are reproducible, but the specific concentration ranges quoted in Tables 4 and 7 are collated from heterogeneous sources and should be regarded as indicative until measured on the matched samples of Section 11.

A second set of conclusions is plausible and is supported by mechanism and by partial evidence but not by direct NMR measurement on sourdough. The anti-staling effect of sourdough is well documented at the level of texture but its attribution to specific mechanisms—dextrins, exopolysaccharides, arabinoxylan solubilization, acid-modified gluten—has not been resolved by the relaxometric and solid-state measurements that could resolve it, and the relaxometric response of dough to sourdough acids, metabolites, and polymers during proofing has not been reported systematically. The DOSY observation of arabinoxylan size reduction during fermentation is recent, is based on a limited set of strains and conditions, and includes deviations from the general trend that the authors themselves flag as requiring further work.[26] The co-metabolite authentication criterion of Table 7 is derived from the biochemistry and is consistent with the qPCR findings of Pontonio et al., but it has not been validated across flours, processes, and dosages of sourdough ingredients in a published study, and a laboratory deploying it should first calibrate it on breads

of known history.[47] The benchtop feasibility estimates of Section 9.5 are extrapolated from the resolution and sensitivity of 60–80 MHz instruments on comparable matrices and should be verified on sourdough extracts before any at-line claim is made.

A third set of claims that circulate in the sourdough literature and in commerce is speculative or is outside the reach of NMR and should not be made on the basis of NMR data. The reduction of gluten immunoreactivity by sourdough fermentation has been demonstrated only with selected strains, prolonged fermentation, and added fungal proteases, and no bread made from wheat by a conventional sourdough process has been shown to be safe for individuals with celiac disease; ^{1}H NMR of an extract measures free amino acids and peptides and says nothing about the immunoreactive gliadin epitopes that remain in the insoluble fraction.[22] The odor-active volatiles that distinguish sourdough bread are present at concentrations below the limit of quantification of NMR and require gas chromatography–olfactometry. The enantiomeric composition of lactate, which is a genuine microbial fingerprint, is inaccessible to achiral NMR. Claims of improved mineral bioavailability, of reduced glycemic response, and of prebiotic benefit are supported by mechanism and by specific studies but depend on the flour, the process, and the consumer, and an NMR measurement of phytate speciation or of lactate content is evidence of the chemical precondition for such an effect, not of the effect.[28,32] Finally, the sourdough metabolite ranges reported across the literature depend on the extraction and the basis of expression to an extent that is rarely acknowledged; a laboratory comparing its own results with published values should reconstruct the basis before concluding that a sample is atypical.

An expert reviewer, or an opposing expert in an authentication dispute, would seize on three points. First, that the concentration thresholds in Table 7 are not standardized and that a product could be argued to fall on either side of them; the answer is that the criterion is a pattern applied against reference samples, and the laboratory should hold those reference samples and their spectra. Second, that a generously dosed sourdough ingredient reproduces the pattern of a starter-leavened bread; the answer is that succinate and residual maltose distinguish the two in most cases and that the laboratory should report the intermediate cases as intermediate. Third, that NMR alone does not establish the enantiomeric composition of the lactate or the isotopic origin of the acetate; the answer is to obtain those measurements when the finding will be contested. None of these is a weakness of the method; each is a limitation that should be written into the report before it is discovered by the other side.

13. Conclusions

Sourdough is a chemically distinct system from yeast-leavened bread at every stage from starter to stored loaf, and the distinction is written into the composition of the dough in a form that NMR spectroscopy reads with unusual completeness. The heterofermentative lactobacilli that dominate traditional starters convert the maltose released by the flour's own amylases into lactate, ethanol or acetate, and carbon dioxide, reduce fructose to mannitol when they use it as an electron acceptor, release glucose that feeds maltose-negative yeasts, depolymerize the gluten by thiol exchange and by activating the cereal proteases, convert arginine to ornithine, decarboxylate glutamate to γ-aminobutyrate, hydrolyze phytate through the acid activation of the cereal phytase, solubilize and shorten the arabinoxylans, and, when sucrose is available, synthesize dextrans and levans; the yeasts contribute ethanol, carbon dioxide, glycerol, succinate, and the fusel alcohols and their acetates. A single ^{1}H NMR spectrum of a buffered, ultrafiltered aqueous extract quantifies the sugars, acids, alcohols, polyols, and amino acids that these transformations produce or consume, ^{31}P NMR speciates the inositol phosphates, DOSY and ^{13}C NMR characterize the polymers, and the fermentation quotient, the mannitol-to-acetate ratio, the ethanol-to-lactate ratio, and the ornithine-to-arginine ratio derived from the

spectrum are process indices that a bakery or an ingredient supplier can use. Low-field relaxometry follows the mixing, proofing, baking, and staling of the intact dough and crumb through the proton populations of the biopolymers and the water, solid-state ^{13}C NMR follows the loss and return of starch order, and imaging follows the gas cells; these are the methods that can convert the anti-staling and crumb-structure claims made for sourdough from assertions into measurements. For the authentication of sourdough bread, the acid content is insufficient because acid can be added; the co-metabolite pattern of mannitol, ornithine, γ-aminobutyrate, amino acid enrichment, sugar depletion, and low succinate is the defensible criterion, and it should be supplemented by an enzymatic lactate enantiomer assay and, where contested, by isotopic analysis of the acetate. The program set out in Section 11 would establish the reference data on which these applications depend, and the results of its execution will be reported in a subsequent version of this paper.

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Summary

This white paper has treated the chemistry of sourdough and the application of NMR spectroscopy to it from the standpoint of an analytical laboratory. The sourdough ecosystem was described in terms of the current taxonomy (*Fructilactobacillus sanfranciscensis*, *Maudiozyma humilis*) and the Type 0–IV classification, with the stiff and liquid levains, refreshment regimes, dough yields, temperatures, and flour extraction rates that select the microbiota explained as ecological drivers. The flours available for sourdough—bread wheat at several extraction rates, rye, durum, spelt, einkorn, emmer, barley, oat, maize, rice, sorghum, millet, teff, buckwheat, quinoa, amaranth, and the legume flours—were compared through the compositional attributes that govern fermentation: fermentable sugars and amylolytic potential, protein type, arabinoxylans and β-glucans, fructans, phytate, lipids, endogenous enzymes, and buffering capacity. The fermentation biochemistry was developed from the maltose phosphorylase and phosphoketolase pathway through the electron-acceptor chemistry that yields mannitol and acetate, the fermentation quotient, homofermentative and yeast metabolism, acidification and acid speciation, proteolysis and thiol exchange, the arginine deiminase and glutamate decarboxylase pathways, phytase, exopolysaccharide synthesis, antifungal metabolites, and flavor formation, and the process of mixing, fermentation, proofing, baking, cooling, and staling was described and contrasted with straight-dough baker's-yeast bread, in which succinate is the dominant acid, the pH stays near 5.5, proteolysis is minimal, and staling proceeds faster.

The NMR review matched five modalities to the sample states and questions of sourdough science. High-field solution ^{1}H NMR of extracts, with a tabulated assignment of the diagnostic resonances and a quantification protocol, was presented as the primary window on the metabolites and their process indices; ^{13}C, two-dimensional, and diffusion-ordered spectroscopy were presented for the polymers, with the recent demonstration that fermentation reduces the size of the water-extractable arabinoxylans; ^{31}P NMR was presented as the definitive speciation method for phytate and its degradation products; HR-MAS and ^{13}C CP/MAS solid-state NMR were presented for intact flour, dough, crumb, and crust, including the loss of A-type starch crystallinity on baking, its partial retention in the crust, and the return of B-type order on staling; low-field relaxometry was presented for the proton populations of dough and bread and their evolution through mixing, proofing, gelatinization, and staling; and imaging was presented for the gas cells and water distribution, with the acknowledgment that no sourdough-specific imaging study exists. A co-metabolite criterion for distinguishing starter-leavened sourdough bread from yeast bread acidified with added acids or containing a sourdough ingredient was set out, with its limitations, and a critical assessment separated the established conclusions from the plausible ones and identified the claims—gluten immunoreactivity, volatile aroma, lactate enantiomers, and health effects—that NMR cannot support. A sampling and measurement program for Process NMR Associates, executable on 300 MHz and 60 MHz instrumentation with solid-state measurements where available, was proposed, and its subsections for experimental results were reserved for version 1.1. Sixty references, each verified against the CrossRef registry, support the text.