Metabolomic and Proteomic Analysis of Biochemical Changes in Dough During Frozen Storage

Quentin Herrera
School of Electrical Engineering and Computer Science, Oregon State University, Corvallis, OR, USA.
contactquentin@oregonstate.edu

Navin Bandra
Department of Computer Science, University of Alabama at Birmingham, Birmingham, AL, USA.
chandra798@uab.edu

Abstract

Frozen dough systems are widely used in industrial baking because they separate production from consumption and reduce waste, yet freezing and frozen storage generate biochemical alterations that compromise gas retention, crumb structure, and product consistency. This paper presents a systems-oriented examination of metabolomic and proteomic approaches to characterize these alterations. It argues that the frozen dough matrix should be understood as an integrated biochemical system in which ice formation, water migration, yeast cryoinjury, gluten depolymerization, lipid oxidation, and metabolite leakage interact across molecular scales. Metabolomic profiling captures shifts in fermentable sugars, amino acids, organic acids, membrane lipid components, and stress-related metabolites, while proteomic analysis identifies changes in gluten protein aggregation, redox-sensitive thiol status, proteolytic fragments, and enzymes involved in dough maturation. The analytical value of these methods depends not only on instrument resolution but also on data architecture, workflow automation, spectral libraries, and statistical inference. The paper examines structural trade-offs in cryoprotection and dough formulation, including the use of polysaccharide sol-gel transitions to stabilize interfacial water. It further addresses governance, reproducibility, cold chain infrastructure, environmental burdens, and equity implications. Robustness across wheat varieties, yeast strains, and freezing equipment conditions remains a critical challenge for translating omics-derived markers into routine quality management. By connecting molecular insights to system design and policy, the paper contributes an interdisciplinary framework for next-generation frozen dough diagnostics and sustainable bakery operations.

Keywords

metabolomics; proteomics; frozen dough; gluten network; cryoprotection; multi-omics integration; cold chain governance.

1. Introduction

Frozen dough is a complex biochemical system whose industrial value arises from the decoupling of dough production from baking and retail. This decoupling enables centralized manufacturing, lower in-store labor requirements, and reduced discarding of unprepared products. However, the freezing process and subsequent frozen storage expose the dough to physicochemical stresses that alter fermentation capacity, water distribution, and viscoelastic behavior. Ice crystal growth damages yeast membranes and disrupts the gluten network, while

recrystallization during temperature fluctuation accelerates moisture migration and textural deterioration. Classical empirical methods such as farinography, extensiography, and bake tests provide useful aggregate measures, but they rarely reveal the molecular events that precede failure. A systems-level account of frozen dough quality therefore requires metabolomic and proteomic methods that can resolve biochemical changes in yeast, gluten, starch-associated proteins, and the soluble metabolite pool [1], [2], [3].

The intellectual foundation of metabolomics rests on measuring the collective low-molecular-weight chemical constituents of a biological or food matrix as a function of time and environmental condition [4]. In frozen dough, the metabolome includes sugars, organic acids, amino acids, nucleotides, lipids, and secondary products of oxidative or enzymatic reactions. Proteomics extends this view toProteomics extends this view to the protein complement and its post-translational modifications, degradation products, and interaction states [5]. Together, these omics layers provide a molecular foundation for understanding the empirical changes observed in frozen dough. Whereas metabolomics captures the small-molecule environment that supports yeast metabolism and affects flavor precursors, proteomics reveals how the structural proteins responsible for gas retention are gradually modified by ice-associated stress. Because the dough matrix is not a biological cell line or a homogeneous solution, the application of metabolomics and proteomics to frozen dough requires careful adaptation of extraction protocols, spectral acquisition workflows, and statistical modeling. The analytical challenge is compounded by the presence of starch granules, denatured gluten aggregates, lipid-protein complexes, yeast cells, and variable water pools. A systems-level interpretation therefore treats the metabolome and proteome as coupled layers of the same frozen dough infrastructure. This perspective also connects molecular measurements to practical decisions in formulation, cryoprotection, cold chain management, and equitable access to frozen bakery technologies.

2. Metabolomic Architecture of the Frozen Dough Matrix

The metabolome of wheat flour dough is derived from multiple sources, including the wheat kernel, added process water, yeast metabolites, exogenous enzymes, and reaction products generated during mixing, resting, freezing, and thawing. The primary metabolite classes relevant to frozen dough quality include fermentable sugars such as maltose, glucose, and fructose; organic acids such as lactic, acetic, succinic, and citric acids; free amino acids; nucleotide derivatives; phospholipid membrane components; and lipid oxidation products. During frozen storage, solute concentration in the unfrozen water phase can rise dramatically, altering reaction kinetics and shifting microbial activity. Conventional fermentation analysis may measure total gas production, but metabolomic profiling can resolve which substrates are depleted and which inhibitory metabolites accumulate before fermentation failure becomes evident. Nuclear magnetic resonance spectroscopy and mass spectrometry platforms allow comparative quantification of these metabolite pools across storage time and temperature conditions [6]. Liquid chromatography-mass spectrometry has been particularly valuable for identifying non-volatile polar metabolites, whereas gas chromatography-mass spectrometry can detect volatile organic compounds and lipid-derived fragments that influence aroma and crumb structure.

A central observation from metabolomic studies is that freezing does not simply slow biological activity; it selectively inhibits some pathways while allowing others to continue. For example, yeast invertase and amylase activity may continue to release fermentable sugars from damaged starch or damaged yeast cells even when glycolysis is partly suppressed. This

imbalance can produce a latent pool of fermentable sugars that triggers rapid gas production during later proofing, resulting in uneven crumb and crust color. In parallel, free fatty acids released from lipid membranes can form complexes with starch and gluten fractions, altering surface activity and gas cell stability. Because metabolomic data contain many correlated variables, multivariate models are needed to distinguish storage-related shifts from biological or flour batch variance. The integration of metabolomic features with conventional bulk parameters such as extensibility and water activity exemplifies a hybrid diagnostic strategy that is more sensitive than either approach alone.

At the same time, metabolomic workflows in dough research must contend with high sample viscosity, low analyte abundance, and matrix effects from starch and protein. Extraction solvents that work efficiently in plant tissues may be less effective in partially frozen dough matrices, where solutes are spatially segregated by ice structures. The choice of quenching method also affects metabolite stability, since slow freezing during sample preparation can reproduce the very ice damage that the experiment seeks to measure. Robust metabolomic protocols therefore include internal standards, quality control samples, and normalization strategies that account for variable water content. Cryoprotective strategies can be informed by observing the structural basis of sol-gel transitions in plant polysaccharide fractions [7]. Such transitions may reduce ice crystal mobility and stabilize the unfrozen phase, thereby altering the metabolite leakage profile measured by mass spectrometry.

3. Proteomic Alterations and Gluten Network Integrity

The gluten protein network is the principal structural scaffold of wheat dough, and its integrity determines the ability of the dough to retain carbon dioxide during proofing and early baking. Frozen storage induces several proteomic changes, including depolymerization of glutenin macropolymers, oxidation of free thiol groups, release of low-molecular-weight peptides, and aggregation of gliadin fractions [2], [3]. The relative abundance of high-molecular-weight glutenin subunits is often correlated with dough strength, but proteomic analysis reveals that this relationship is mediated by disulfide bond topology rather than simple subunit quantity [17]. During freezing, ice crystal growth can physically rupture the hydrated protein matrix, while freeze-induced concentration of salts and organic acids can promote non-disulfide covalent cross-links. The resulting balance between physical breakage and chemical cross-linking explains why some frozen doughs become more extensible while others become more resistant but less extensible after storage [9].

Proteomic workflows in dough research typically combine protein extraction, enzymatic digestion, and tandem mass spectrometry. The identification of oxidative modifications, such as carbonylation and oxidation of methionine and cysteine residues, provides direct evidence of the redox stress that accompanies ice recrystallization. The activity of endogenous proteases can be altered by freezing, leading to a different peptide fingerprint even when the total protein content remains unchanged [10]. In some cases, insoluble gluten aggregates resist conventional extraction, requiring sequential solubilization strategies to avoid underrepresentation of the most functionally important components. Recent work has also shown that starch-associated proteins and lipid-binding proteins are affected by frozen storage, indicating that the proteome extends beyond the gluten fraction to include minor proteins with functional roles at interfaces [8]. Ribotta and colleagues demonstrated that freezing and frozen storage modify starch gelatinization and retrogradation properties, suggesting that protein-starch interactions should be considered alongside gluten-specific changes [8].

The interpretation of proteomic data in frozen dough requires a systems-level view of network formation and failure. A decrease in total glutenin abundance may be less predictive than a change in the ratio between extractable and unextractable protein fractions, because the latter reflects the degree of covalent cross-linking and polymerization. Shotgun proteomics offers a broad inventory of peptides, but targeted proteomics can quantify selected marker peptides with higher precision. For example, peptides derived from glutenin subunits can serve as indicators of subunit-specific degradation or aggregation. However, the conversion of peptide signals into functional predictions remains difficult because the viscoelastic properties of dough are emergent properties of the whole network. This challenge is analogous to the problem of predicting infrastructure resilience from component-level stress data: individual molecular markers are informative, but their significance depends on network architecture and environmental history.

4. Data Integration, Reproducibility, and Workflow Governance

The effective use of metabolomic and proteomic data in frozen dough research depends on data architecture, spectral alignment, statistical inference, and reproducibility. Large-scale omics workflows generate thousands of features from each sample, and the reliability of these features depends on instrument calibration, retention time alignment, and database coverage [11]. A metabolomic data matrix may contain missing values because some metabolites fall below detection limits in degraded samples, while others are masked by matrix interferences. Similar challenges arise in proteomics, where peptide identification rates vary with protein extraction efficiency and database completeness. The use of open spectral libraries and community-standard reference materials improves comparability across laboratories, but many dough-specific metabolites and wheat peptides remain underrepresented in public databases. Data governance therefore becomes a first-order issue rather than an administrative afterthought. The FAIR principles for scientific data management provide a useful framework for ensuring that omics datasets are findable, accessible, interoperable, and reusable [12].

Reproducibility in frozen dough omics is fragile because the input material is itself heterogeneous. Wheat varieties differ in protein composition, enzyme activity, and starch characteristics, while flour milling methods alter particle size and damaged starch content. The freezing rate, storage temperature, and temperature fluctuation history further influence ice morphology and solute redistribution [20]. Without explicit metadata recording these variables, metabolomic and proteomic datasets cannot be meaningfully compared across institutions. The adoption of reporting standards, such as minimum information about a metabolomics experiment, would enable meta-analyses that link molecular markers to storage outcomes. Governance mechanisms might include shared repositories for dough omics data, standardized freezing protocols, and interoperable ontologies that describe processing conditions. Such mechanisms are particularly important when molecular markers are intended to guide quality control decisions in industrial settings, where false positives or non-transferable models can create economic and food waste burdens.

The integration of metabolomic and proteomic features requires careful statistical treatment to avoid spurious correlations and overfitting. Because omics datasets typically contain more variables than samples, internal cross-validation and external validation on independent dough batches are essential. Data fusion methods can be used to identify metabolite-protein pairs that jointly predict bake quality, but these models must be interrogated for mechanistic plausibility. A metabolite that predicts loaf volume in one dataset may function only as a proxy for water content or yeast health, not as a direct cause of changed functionality.

Systems-level inference should therefore combine statistical association with biochemical reasoning, drawing on known pathways of yeast stress response and gluten redox chemistry. The development of transparent, reproducible analytical pipelines represents a governance challenge because proprietary industrial data and open academic data may have different incentives and access regimes. Nevertheless, the long-term benefit of shared reference datasets outweighs the short-term costs of coordination.

5. Structural Trade-offs in Cryoprotection and Formulation

Frozen dough formulations involve multiple trade-offs among water availability, gluten hydration, yeast viability, and ice crystal control. Cryoprotective additives such as sugars, polyols, hydrocolloids, and antifreeze proteins lower the freezing point or modify ice crystal habit, but they also alter dough rheology and final bread texture. The desired cryoprotective effect is often achieved at the expense of longer proofing time, increased osmotic stress on yeast, or altered crumb structure. Polysaccharide fractions with sol-gel transition behavior can stabilize the unfrozen water phase and reduce ice recrystallization without necessarily increasing solute concentration to growth-inhibiting levels [7]. The structural basis of such transitions involves the formation of hydrated networks that slow water diffusion, yet the same networks may restrict gas cell expansion during proofing. Thus, the cryoprotective strategy must be evaluated as a system-level intervention rather than as an isolated additive response.

Hydrocolloids and other stabilizers interact with the gluten network and starch granules in ways that metabolomic and proteomic methods can illuminate. A cryoprotectant may preserve gluten extractability but simultaneously change the metabolite pool available for yeast fermentation because it alters water partitioning. Inulin, for example, has been investigated for its effects on water state and gluten during frozen storage, with evidence that its prebiotic and humectant properties are not separable from its influence on protein hydration [14]. Proteomic analysis can reveal whether a cryoprotectant stabilizes specific glutenin subunits or reduces proteolysis, while metabolomic profiling can determine whether the additive alters yeast byproduct formation. These molecular observations help formulators distinguish cryoprotective effects from simply delaying the expression of damage. The structural trade-off is not merely chemical but also economic: high-value polysaccharides may improve quality but raise ingredient costs, while inexpensive sugars may increase sweetness or browning beyond acceptable limits.

The deployment of metabolomic and proteomic data in formulation science also has implications for robustness across wheat varieties and processing plants. A cryoprotectant that stabilizes a high-protein wheat dough may be less effective in a weak flour with different glutenin-to-gliadin ratios. Similarly, yeast strains vary in their tolerance to cold shock and osmotic stress, and these differences are reflected in metabolite leakage profiles [16]. A systems approach would use omics markers to match cryoprotective formulations to specific flour and yeast combinations, reducing the current reliance on trial-and-error reformulation. This strategy parallels precision agriculture and personalized nutrition, where molecular data enable interventions tailored to the biological variability of the system. The industrial adoption of such precision freezing protocols depends on rapid analytical turnaround, robust predictive models, and training for quality control personnel.

6. Cold Chain Infrastructure, Environmental Sustainability, and Equity

The quality of frozen dough is inseparable from the infrastructure that maintains temperature stability from manufacturing to retail. Cold chain interruptions can produce partial thawing and recrystallization, which cause greater damage than continuous freezing at a constant temperature. Metabolomic and proteomic markers can function as sentinel indicators of cold chain abuse, revealing molecular damage before visible or rheological changes appear. However, the deployment of such markers requires reliable temperature logging, efficient data transmission, and decision support systems that integrate molecular, environmental, and logistical data. The cold chain governance problem is therefore both technical and organizational, involving suppliers, distributors, and retailers with varied capabilities and incentives [19]. A monitoring strategy that depends on expensive omics analyses will not be sustainable in low-margin bakery operations; thus, marker-based approaches must be translated into affordable sensors or simplified assays.

Freezing is an energy-intensive preservation technology, and the expansion of frozen dough markets has environmental consequences that extend beyond direct electricity consumption. Refrigerant leakage, packaging waste, and the food waste avoided by frozen formats must be weighed together in any sustainability assessment [13]. Metabolomic and proteomic methods can contribute to environmental sustainability by identifying molecular thresholds for acceptable quality, enabling shorter storage times or higher storage temperatures without excessive quality loss. If a metabolomic marker shows that dough remains functionally stable at minus 15 degrees Celsius for a shorter period rather than minus 20 degrees Celsius for a longer period, the cold chain could be optimized for energy efficiency. However, such decisions require a rigorous understanding of quality degradation kinetics and the uncertainty associated with marker-based predictions. The infrastructure trade-off between energy use and product consistency is analogous to the trade-off between refrigeration intensity and gluten network stability: there is no single optimum, only a strategy that balances competing system demands.

Equity considerations are often overlooked in advanced food diagnostics. The benefits of metabolomic and proteomic frozen dough research should not be limited to large industrial bakeries in high-income regions. Small-scale bakeries and food enterprises in low-resource settings may lack access to mass spectrometry, trained personnel, and standardized frozen storage infrastructure. Governance policies could support regional analytical hubs, shared cold storage facilities, and open-source tools that lower the cost of molecular quality assessment. The development of simplified spectral interpretation workflows, portable near-infrared sensors calibrated to omics markers, and community-managed data repositories can democratize the advantages of systems-level analysis. Without such deliberate design, omics technologies may reinforce existing disparities in food system modernization, concentrating quality gains among actors that can already afford high-resolution diagnostics. The long-term resilience of frozen dough supply chains will depend on inclusive governance that links molecular innovation to infrastructure access and skill development.

7. Conclusion

Metabolomic and proteomic analysis reframes frozen dough quality as an emergent property of interacting molecular, microbial, and physical processes under cold stress. The metabolome reveals how sugar depletion, organic acid accumulation, membrane leakage, and lipid oxidation alter fermentation potential and flavor; the proteome reveals how gluten depolymerization, oxidative modification, and proteolysis degrade gas retention and crumb structure. Neither layer alone can explain the functional variability observed across flours,

yeasts, additives, and storage histories. A systems-level integration of these molecular layers, supported by transparent data governance and reproducible workflows, enables a more mechanistic understanding of frozen dough failure and recovery. The structural trade-offs associated with cryoprotective sol-gel transitions and formulation additives highlight the need for multi-objective design rather than single-factor optimization. Cold chain infrastructure, environmental sustainability, and equity shape the context in which omics-derived knowledge is converted into practical benefit. By treating frozen dough as an integrated biochemical and logistical system, researchers and practitioners can move beyond empirical shrinkage tests toward predictive, resilient, and inclusive frozen bakery quality management. Future work should develop standardized frozen dough omics workflows, validate marker panels across diverse production environments, and build accessible data infrastructure that connects molecular diagnostics to sustainable cold chain governance.

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