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Next-Generation Food Enzymology: From Metagenomic Discovery to AI-Driven Biocatalyst Design

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ABSTRACT: Food enzymology is entering a new era driven by the convergence of metagenomics, artificial intelligence, and synthetic biology. While traditional food processes rely on a limited repertoire of established biocatalysts, metagenomic and multiomics approaches now provide access to vast reservoirs of unexplored enzymatic diversity. Simultaneously, advances in protein structure prediction, functional modeling, and de novo protein design are transforming enzyme discovery from a largely empirical process to a predictive discipline. In this Perspective, we discuss how these technologies will enable the development of tailored biocatalysts for sustainable, precise, and next-generation food processing applications.

KEYWORDS: *food enzymes, metagenome mining, artificial intelligence, protein structure prediction, enzyme engineering*

1. FOOD ENZYMES: FROM EMPIRICAL USE TO RATIONAL OPTIMIZATION

Enzymes shape food quality and process efficiency by catalyzing the transformation of raw biological materials into structured and palatable products. Their compatibility with mild physicochemical conditions makes them particularly suited to food systems, enabling targeted transformations while reducing energy input and chemical additives, thereby supporting more sustainable manufacturing practices. Over the past decades, food enzymology has evolved from empirical use toward increasingly rational and technology-driven deployment. Early applications relied largely on endogenous enzymes or microbial fermentations, where catalytic activities were exploited without detailed molecular understanding. With advances in biochemistry, individual enzymes could be isolated and matched to specific technological functions, enabling their widespread adoption in food processing.[1] Today, enzymes are used across nearly all sectors of food manufacturing, including baking, dairy, beverage, fruit and vegetable processing, and emerging plant-based products. Recent comprehensive reviews highlight how enzyme-assisted processes contribute simultaneously to improved product quality, waste reduction, and process intensification, reinforcing the role of biocatalysis as a central pillar of sustainable food innovation.[2−4]

Concurrently, advances in molecular enzymology and bioprocess engineering have allowed researchers to rationally optimize the catalytic performance. Strategies including strain improvement, recombinant expression, immobilization technologies, and semirational protein modification have expanded operational stability and broadened application windows. These advances have enhanced resistance to temperature, pH, and complex food matrices, allowing enzymes to function reliably under industrial constraints while improving yields and reducing processing times.[5] Nevertheless, despite substantial technological refinement, the enzymatic repertoire that is currently exploited in food systems remains limited. Most processes rely on a set of historically established enzyme families that have been repeatedly optimized. As food matrices become more complex, for example, with the use of alternative proteins or novel carbohydrates, this limited catalytic diversity becomes a constraint for innovation. Recent assessments suggest that next-generation applications will require more selective and multifunctional biocatalysts.[6,7] Recent advances in metagenomics, protein design, and artificial intelligence (AI) are enabling the identification and development of new biocatalysts. Collectively, these developments indicate that food enzymology is entering a new era (Figure 1).

2. THE RISE OF METAGENOMICS AND INTEGRATED OMICS

Over the past two decades, high-throughput sequencing and systems biology have profoundly reshaped how enzymes are discovered and characterized (Figure 1). Classical enzyme discovery relied largely on the cultivation of microorganisms followed by biochemical screening, an approach that restricted exploration to the small fraction of microbial diversity that can be grown under laboratory conditions. Because most environmental microorganisms remain uncultivable, a large portion of Earth's catalytic potential has long remained inaccessible through purely culture-based approaches. Metagenomics

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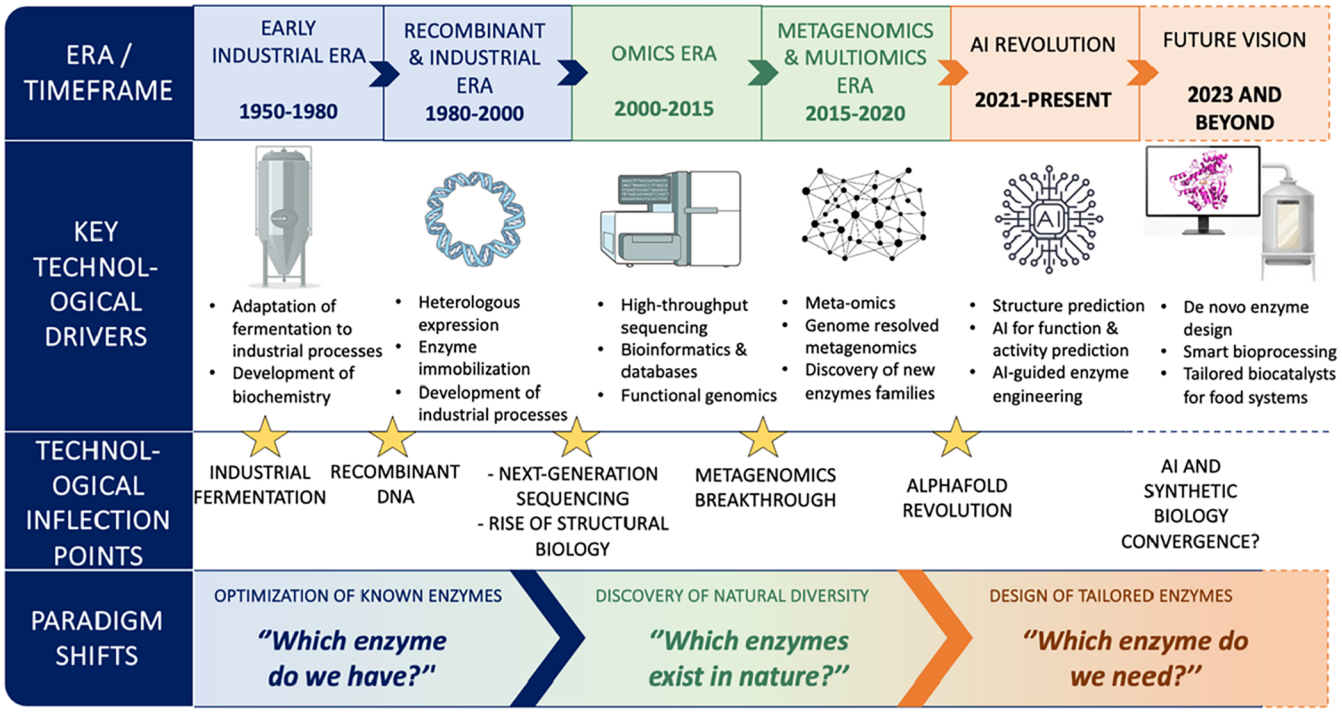

Figure 1. Historical context and perspectives concerning the future of food process enzymology.

emerged as a decisive breakthrough by enabling direct sequencing of genetic material extracted from complex ecosystems, thereby bypassing cultivation and opening access to vast reservoirs of enzymatic diversity.[8−10] Early functional metagenomics relied on activity-based screening of expression libraries but remained experimentally intensive. Rapid improvements in sequencing depth, assembly algorithms, and annotation pipelines have progressively shifted discovery toward sequence-driven strategies, in which millions of predicted open reading frames can be computationally prioritized before targeted validation. In parallel, the emergence of long-read sequencing technologies and improved genome reconstruction methods has enabled genome-resolved metagenomics and the recovery of increasingly complete metagenome-assembled genomes (MAGs). These advances provide access to the genomic repertoires of previously uncultivated microorganisms. By restoring their genomic context, researchers can better interpret enzyme functions and establish clearer links between catalytic repertoires and ecological adaptation.[11−13]

Beyond metagenomics alone, the integration of complementary omics layers (metatranscriptomics, metaproteomics, and metabolomics) has further refined the ability to link genes to their functions. These approaches provide contextual information on which genes are actively expressed, which proteins accumulate, and which metabolic conversions occur under specific environmental or processing conditions.[14,15] For food sciences, one of the most compelling advantages of omics-based discovery lies in the ability to target ecologically relevant niches. Enzymes derived from food and fermentation microbiomes have evolved to operate efficiently at mild temperatures, fluctuating pH, high osmotic pressure, and chemically complex substrates. These conditions are advantageously close to those encountered in industrial food processes.[16] Similarly, enzymes sourced from extreme environments often exhibit enhanced stability or unusual catalytic features arising from identifiable structural adaptations, including reinforced electrostatic interaction networks, increased hydrophobic packing, or more rigid protein architectures.[17,18] Such features can confer resistance to temperature, salinity, or pH extremes while maintaining catalytic activity. This ecological preadaptation increases the likelihood that newly identified enzymes will display desirable technological traits without extensive engineering. More broadly, foodomics, which integrates genomic, proteomic, and metabolomic information to improve processing performance, quality control, and safety, highlights the expanding role of omics technologies across the food innovation pipeline.[19] Conceptually, metagenomics also shifts enzyme discovery from an organism-centric to a function-centric paradigm. Rather than focusing on a limited set of model species or industrial strains, discovery efforts can now search directly for catalytic motifs, domain architectures, or specific stability or reaction signatures across entire communities. These approaches also enable the identification of enzymes that are active under specific pH, temperature, or inhibitory conditions characteristic of food matrices.

Carbohydrate-active enzymes are among the most prominent outcomes of this strategy (Table 1). Gut and food-associated microbiomes constitute rich reservoirs of glycoside hydrolases capable of processing complex dietary polysaccharides and oligosaccharides. For instance, a β-1,2-galactosidase from *Bacteroides xylanisolvens* exhibits strict linkage specificity toward uncommon glycans, while α-L-fucosidases from infant gut metagenomes enable the synthesis of fucosylated oligosaccharides such as fucosyllactose.[20,21] Such discoveries directly support the rational production of prebiotics and the targeted modification of dietary fibers and carbohydrates. These enzymes also illustrate how ecological specialization can translate into highly tuned catalytic architectures. For example, the β-1,2-galactosidase from *B. xylanisolvens* possesses an active-site geometry adapted to uncommon β-1,2-linked glycans, while engineered α-L-fucosidases demonstrate how

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Table 1. Examples of Metagenomic Enzymes of Biotechnological or Food Relevance Recently Characterized in the Literature

catalytic function	source ecosystem/microbiome	enzyme (family or activity)	substrate or reaction	food/biotech relevance	key technological properties and mechanisms	references
Carbohydrate-active enzymes	Human gut (*Bacteroides xylanisolvens*)	β-1,2-galactosidase (GH family)	Complex dietary glycans	Prebiotic design, fiber modification, selective carbohydrate tailoring	Exhibits an active site adapted for β-1,2-galactooligosaccharides hydrolysis	21
	Infant gut microbiome	α-L-fucosidases (GH29)	Fucosylated oligosaccharide synthesis	Human milk oligosaccharide production; functional ingredients	Transfucosylation efficacy improved by tailored point mutations in the active site	20
	Bifidobacterium spp.	α-L-rhamnosidase	Deglycosylation of plant glycosides (e.g., ginsenosides, flavonoids)	Flavor release, bitterness reduction, nutraceutical enhancement	Enhanced activity due to a greater hydrophobicity near the rhamnose-binding pockets	22
	Food additive-degrading microbiome	Xanthan-degrading CAZymes	Hydrocolloid/polysaccharide breakdown	Texture control; digestion behavior of gums	Activity on complex exopolysaccharides	31
	Soda lake metagenome	GH3 β-glucosidase	Glycosidic bond cleavage in biomass	Biomass valorization; carbohydrate processing	Halo/thermostable; tolerant to harsh conditions due to numerous electrostatic interactions in the enzyme core	18
	Engineered enzyme (structure-guided)	β-Fructofuranosidase	Fructooligosaccharide synthesis	Prebiotic sweeteners; functional carbohydrates	Point mutations in the active site improved transfructosylation yield, comparable to commercial enzymes	32
Hydrolytic enzymes	Environmental metagenome	γ-type carbonic anhydrase → engineered esterase	Ester hydrolysis	Aroma precursor release; lipid modification	Hyperthermostable scaffold and increased esterase activity due to a heptapeptide insertion at the active site loop and two-point mutations	33
	Extremophile genome mining	Hyperthermostable amidase	Amide bond hydrolysis	Robust biocatalysis; harsh processing conditions	High thermal stability and active site adapted to diverse nylon-type substrates	34
	Human saliva metagenome (engineered)	PET hydrolase	Polyester depolymerization	Circular bioeconomy; packaging waste valorization	Enhanced catalytic efficiency after engineering loop variants using a high-throughput mass-spectrometry screening platform and molecular dynamics	35
	Bacillus strain isolated from ricotta	Endopeptidase	Generates an antimicrobial peptide after hydrolysis of casein	Eco-friendly preservative for dairy products	Active site adapted to a polypeptide substrate	25
	Dairy industry stabilization ponds	Proteases	Whey proteins hydrolysis	Production of value-added products from dairy industrial byproducts	Proteolysis activity comparable to commercial proteases	24
	Diverse environments	Lipases B	Fatty acids ester hydrolysis	Esterification, transesterification and acidolysis	Structure-based screening using the commercial CalB as template led to identification of high thermotolerant new candidates with better optimal temperature (55 °C)	27,28
Transglutaminases	*Streptomyces mobaraensis*	Microbial transglutaminase (MTG)	Aminoacyltransferase reaction producing covalent bonds between protein molecules	Increase the viscosity, elasticity, and water-retaining ability of foods	Highly active and stable MTG mutant obtained by a computer-aided design and a cavity strategy to adapt it to the gelation of soy protein isolate	29,30
Oxidoreductases	Human gut microbiota	Reductases/sulfotransferases	Phenolic and steroid metabolites	Modulation of phenolics affecting color, astringency, aroma stability	Broad substrate scope; small-molecule specificity	36
	Thermoalkaliphilic bacterium from a hot spring	Laccase	Decolorize synthetic dyes using acetosyringone as redox mediator	Eco-friendly oxidases for multiple industrial purposes	High specific activity at 70 °C and maintain 60% activity after 2 h at 60 °C	37

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

subtle modifications of catalytic residues can substantially improve transglycosylation efficiency.[20,21] Similarly, α-L-rhamnosidases from *Bifidobacterium* convert glycosylated plant metabolites into more bioactive or flavor-active forms.[22] Because these compounds strongly influence sensory properties in beverages and plant-based foods, enzymes with defined specificity toward these conjugates offer attractive tools for precision modulation of sensory properties without extensive reformulation.[23] Beyond carbohydrate-active enzymes, metagenomic and genome-mining approaches are increasingly expanding the repertoire of hydrolytic and cross-linking enzymes relevant to food processing. Novel proteases identified from dairy-associated ecosystems have shown hydrolytic performances comparable to commercial preparations (HPLC activity profiles similar to the Novozyme 11028 protease). Moreover, they facilitate the valorization of protein-rich byproducts[24] and the generation of antimicrobial peptides from food proteins.[25] Lipases discovered through structure-guided mining of environmental sequence databases further demonstrate the potential of these approaches for lipid modification, with several candidates displaying enhanced thermotolerance compared with benchmark industrial enzymes (60% remaining activity at 70 °C while the commercial Lipozyme CalB is inactive at this temperature). Such improvements are often associated with increased structural rigidity and stabilization of the catalytic scaffold, enabling activity at temperatures that would compromise conventional biocatalysts.[26−28] Furthermore, advances in enzyme engineering are of great interest for microbial transglutaminases, whose ability to form covalent protein cross-links is widely exploited to improve texture, water-holding capacity, and gelation properties. Recent structure-guided optimization strategies based on cavity optimization have generated variants with enhanced activity toward plant proteins with respect to the wild-type enzyme (14.45-fold increase in activity accompanied by a T_m value elevation of 1.2 °C), highlighting their relevance for emerging plant-based foods.[29,30] Together, these examples illustrate that the impact of metagenomics and computational discovery provides access to a broad spectrum of catalytic functions directly relevant to food formulation, preservation, and sustainable bioprocessing.

3. COMPUTATIONAL ENZYME DISCOVERY AND DESIGN: THE FUTURE OF FOOD ENZYMOLOGY

If metagenomics and integrated omics have transformed enzyme discovery into a data analysis problem, the next challenge is to translate this vast sequence space into actionable functional knowledge. Addressing this gap increasingly relies on computational and AI-based approaches capable of predicting three-dimensional protein structures at atomic resolution, inferring function, and guiding rational design (Table 2). These advances, including AlphaFold and RoseTTAFold, now enable highly accurate structure prediction from sequence.[38,39] For enzyme discovery, particularly in metagenomic contexts where thousands to millions of candidates lack experimental characterization, this capability dramatically lowers the structural barrier. High-confidence models now enable systematic identification of catalytic residues, active-site architectures, substrate-binding pockets, and domain organizations across entire enzyme families. Recent studies also show that predicted models can provide insights into enzyme conformational flexibility relevant for catalysis and engineering.[40−43] This additional information is

Table 2. Representative AI-Based Tools Supporting Enzyme Discovery, Characterization, and Design for Food Biotechnology

discovery stage	tool	algorithm	input data	output/prediction	relevance for enzyme discovery and food applications	key reference
Structure prediction	AlphaFold	Transformer-based deep neural network with attention mechanisms and evolutionary information from multiple sequence alignments	Amino acid sequence	High-accuracy 3D protein structure	Enables identification of catalytic residues, substrate-binding pockets, and domain organization in metagenomic enzymes	39
	RoseTTAFold	Three-track neural network simultaneously integrating sequence, distance maps, and structural coordinates	Amino acid sequence	3D structural model	Alternative deep-learning structure predictor facilitating structural annotation of large enzyme data sets	38
Functional annotation	DeepEnzyme	Hybrid deep-learning model combining transformer-derived sequence embeddings with graph neural network representations of protein structures	Sequence + structural features	Catalytic turnover number (k_{cat}) prediction	Prioritizes highly active enzyme candidates from metagenomic data sets before experimental screening	44
De novo design	RF Diffusion	Denoising diffusion probabilistic model operating in protein structural space	Target structural constraints	Novel protein backbone structures	Enables design of new catalytic scaffolds potentially tailored to specific food-processing reactions	47
	AlphaFold inversion frameworks	Gradient-based optimization/neural network inversion using AlphaFold as a structure evaluator	Structural constraints	Novel protein folds	Allows generation of new protein scaffolds with potential catalytic functions	48
	ProteinMPNN	Message-passing neural network (graph neural network) for inverse protein folding	Target 3D structure	Amino acid sequence optimized for folding stability	Generates sequences compatible with designed or predicted enzyme structures	46

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

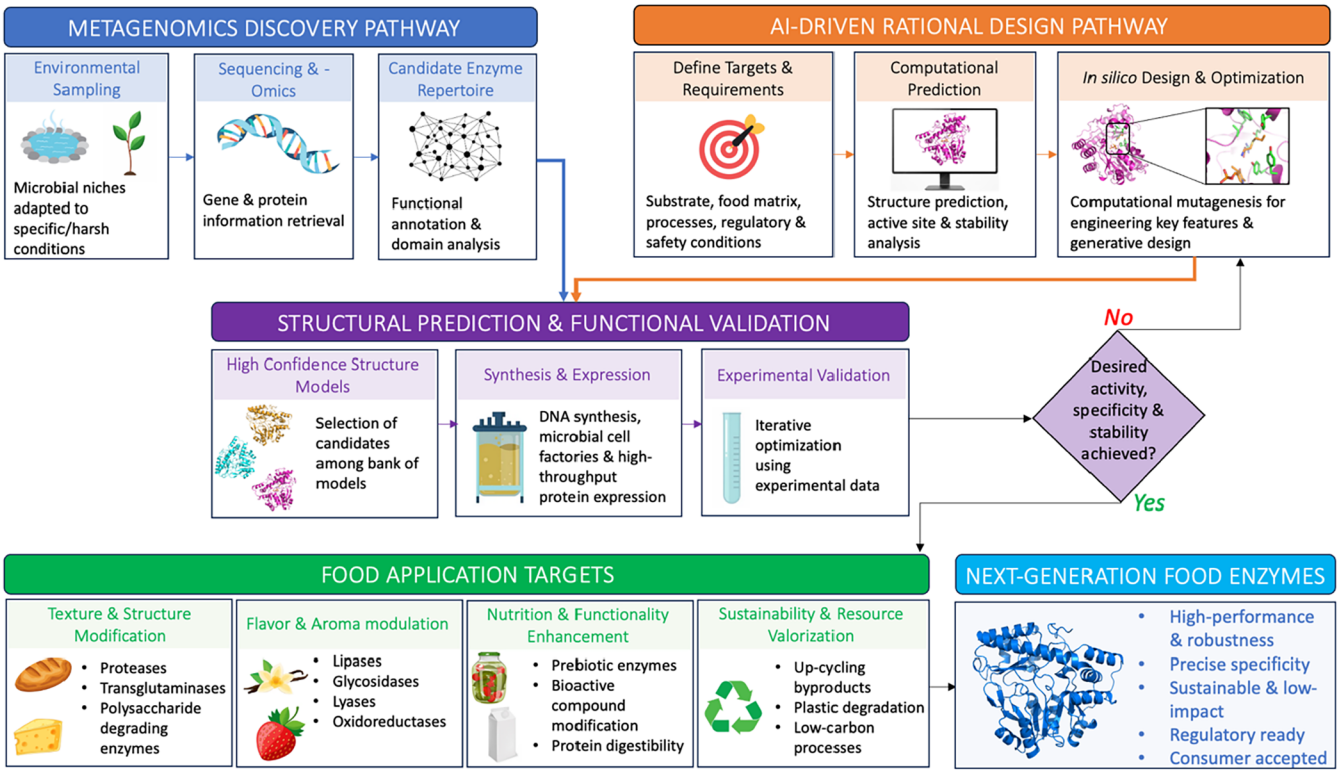

Figure 2. Integrated roadmap for next-generation food enzyme discovery and design.

particularly relevant for engineering substrate specificity, gating motions, or allosteric regulation properties that are often decisive in complex food matrices. Beyond structure prediction alone, deep learning is increasingly used to infer quantitative enzymatic properties directly from sequence and structural descriptors. Deep learning models can also predict enzymatic properties such as catalytic turnover numbers (k_{cat}), enabling prioritization of highly active candidates from large metagenomic data sets.[44] Together, these predictive tools are progressively transforming enzyme discovery from a screening problem into a design problem, where candidate selection, optimization, and experimental validation can be integrated within a single computational workflow (Figure 2).

Once reliable structural or functional predictions are available, rational engineering becomes significantly more tractable. First, emerging technologies accelerate the design–build–test cycle linking computational prediction to experimental validation. Recent advances in synthetic biology and DNA synthesis now enable the rapid fabrication of virtually any metagenomically derived or computationally optimized sequence, independently of its native host. Combined with standardized microbial cell factories, these approaches substantially shorten development timelines and facilitate iterative rounds of computational mutagenesis, active-site remodeling, and stability optimization.[45] Rather than relying on large-scale random screening, enzyme improvement can increasingly be guided by structural information to target residues controlling substrate recognition, catalytic efficiency, or operational stability.

Second, generative AI approaches move beyond optimization of existing enzymes toward the creation of entirely new protein architectures. Models such as RF Diffusion and ProteinMPNN can generate sequences predicted to adopt predefined structural features,[46,47] while inversion of the AlphaFold network has demonstrated that novel protein scaffolds with experimentally validated folds can be designed computationally.[48] These developments suggest a transition from discovering natural catalytic diversity to creating biocatalysts tailored to specific food-processing objectives.[49] In food sciences, these advances are particularly impactful because enzyme performance must be finely tuned to heterogeneous and often harsh conditions as previously described. AI-assisted enzyme engineering frameworks are increasingly being applied to address these constraints, enabling prediction of stability, activity, and mutational hotspots while substantially reducing development time compared with traditional approaches.[50] Despite these transformative advances, important limitations remain. Predicted structures generally represent dominant conformations and may not fully capture dynamics, cofactor interactions, or multicomponent assemblies.[51] Similarly, deep learning models depend on the quality and diversity of their training data set and may struggle with chemistries that are poorly represented in existing data sets. In this regard, atomic-level modeling using molecular dynamics simulations provides a complementary approach, allowing for the exploration of conformational flexibility, interactions, and stability in realistic environments. Nevertheless, experimental validation remains indispensable, particularly in the complex physicochemical environments characteristic of food systems.

Together, AI-driven prediction, functional modeling, and generative design are shifting enzyme discovery from an empirical practice to a predictive and scalable discipline. Looking ahead, computational enzyme discovery is poised to become a central pillar of food biotechnology. By combining large-scale metagenomic exploration with accurate structure prediction, quantitative activity modeling, and *de novo* design, AI provides a powerful bridge between natural enzymatic

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

diversity and application-specific performance requirements. As these technologies mature and become increasingly integrated, food enzymology may evolve from the discovery of naturally occurring catalysts to the design of biocatalytic functions tailored to specific processing, nutritional, and sustainability objectives.

4. TOWARD ADAPTIVE AND DESIGN-DRIVEN FOOD BIOCATALYSIS

Looking beyond current advances in metagenomics and AI, the next phase of food enzymology is likely to emerge from the convergence of digitalization, synthetic biology, and smart processing technologies. The integration of AI across the food chain, from raw material characterization to process control, already illustrates how predictive modeling and data-driven optimization are reshaping food processing workflows.[52] In parallel, synthetic biology offers the possibility of constructing tailored microbial cell factories and engineered enzymatic pathways for the sustainable production of ingredients, flavors, and functional compounds.[53] One foreseeable development is the integration of digitalization, artificial intelligence, and enzyme technology into smart food-processing systems. For example, real-time monitoring of enzymatic reactions coupled with machine-learning models has already been demonstrated for the prediction of glucose concentrations during biotransformations, illustrating how sensor-derived data can support dynamic process optimization and improved control of enzymatic conversions.[54] Additionally, recent work on extremozyme-based biosensors highlights how enzymes adapted to extreme environments can provide enhanced stability and operational robustness compared with conventional biocatalysts.[55] Compared with conventional mesophilic enzymes, extremozymes frequently exhibit substantially broader operational windows, remaining active at temperatures up to 80−95 °C, retaining >50% activity after several hours at 80 °C, and maintaining catalytic performance under highly alkaline or saline conditions. However, current challenges to this approach are rare codon usage, formation of secondary mRNA structures or misfolding during heterologous expression in mesophilic organisms. Current work is focused on the development of new extremophile expression hosts or cell-free systems.[56]

In parallel, synthetic biology is expanding the scope of enzyme-enabled manufacturing by engineering complete metabolic pathways for the production of high-value food ingredients. Recent work has demonstrated the efficient biosynthesis of rare sugars from metabolically engineered microorganisms, highlighting how tailored enzyme cascades can transform low-value feedstocks into functional compounds with applications in nutrition and health.[57,58] More exploratory concepts, including microrobotic systems for targeted sensing or delivery within complex matrices, further highlight how miniaturized technologies could enable precise, localized biocatalysis.[59] Together, these innovations should enable more environmentally efficient processing, improved valorization of bioresources, enhancement of nutritional and organoleptic qualities, and the development of strategies for food preservation and microbial control.[60]

However, these technological advances also raise important scientific and regulatory challenges, particularly regarding the safety assessment of novel enzymes and bioengineered products derived from unconventional or byproduct sources.[61] In the European Union, food enzymes are evaluated primarily on the basis of safety, purity, and intended use, rather than their origin. Enzymes produced by genetically modified microorganisms or obtained through targeted sequence modification are already widely authorized, provided that toxicological and allergenicity assessments demonstrate their safety. Nevertheless, de novo or AI-designed enzymes may increasingly occupy regions of sequence and structural space with no direct natural counterpart, potentially challenging the existing assessment paradigms. Future evaluations may therefore require enhanced structure-based toxicology approaches and improved allergenicity prediction tools. Internationally harmonized frameworks capable of integrating computationally designed proteins while maintaining consumer confidence will likely become an important regulatory priority.[62] In this context, AI-designed or de novo enzymes would likely continue to be assessed under existing risk-based frameworks, requiring a case-by-case evaluation but not being intrinsically prohibited. Regulatory systems are therefore progressively evolving from source-based to function- and risk-based assessments, a transition that will be essential for accommodating future generations of computationally designed biocatalysts while ensuring transparency and consumer protection.

Collectively, these developments suggest that food enzymology is transitioning from a discipline centered on the optimization of naturally occurring catalysts to one capable of discovering, predicting, and ultimately designing enzymatic functions on demand. Importantly, future digital food systems will also depend on novel enzyme components. In this sense, enzymes are not only tools for food transformation but also foundational building blocks of future food systems.

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Notes

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