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Enzymatic Hydrolysis and Microbial Fermentation for Nutritional, Functional and Sustainable Food Processing: A Systematic Review

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ABSTRACT
Enzymatic hydrolysis and microbial fermentation combine the precision of biocatalysis with the metabolic diversity of microorganisms and are increasingly investigated as complementary approaches for improving food quality, nutrient availability, functional properties, and recovery of bioactive compounds. This systematic review synthesizes evidence on their applications in food and food-related matrices, with emphasis on integrated processing. An assembled set of 82 records was identified; nine duplicates were removed, leaving 73 records for eligibility assessment. Four records were excluded, resulting in 69 publications in the screened evidence base. Four additional contextual publications were incorporated during iterative synthesis, giving 73 publications used in the review. Seventeen primary experimental studies examined integrated enzymatic-microbial processing or formulation-level combinations and formed the comparative evidence. Across plant proteins, cereals, fruits, pulses, fermented beverages, microalgae, and food by-products, integrated processing modified protein and cell-wall structures, enhanced the release or accessibility of phenolics and flavonoids, improved protein digestibility or solubility, reduced antinutritional factors, and influenced texture, aroma, and flavour. Outcomes varied according to substrate composition, enzyme class, microbial strain, processing conditions, and treatment sequence. Enzyme-first configurations were advantageous in several cell-wall-rich matrices, whereas fermentation followed by hydrolysis produced pronounced effects in protein systems. Evidence for antioxidant, ACE-inhibitory, and antidiabetic activities was predominantly derived from in vitro assays and should not be interpreted as direct evidence of clinical efficacy. The review highlights the need for standardized enzyme and inoculum levels, treatment sequence, kinetics, bioaccessibility, sensory assessment, safety evaluation, and scale-up economics to support translation into functional foods and sustainable food-processing applications.

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INTRODUCTION

Food systems increasingly require processing strategies that enhance nutrient utilization, functional performance, and bioactive potential while preserving desirable physicochemical and sensory properties and minimizing food-resource losses. Fermentation and enzymatic hydrolysis are well-established biological processing approaches capable of modifying food matrices under relatively controlled conditions. Enzymatic hydrolysis involves the cleavage of specific chemical bonds by selected biocatalysts, whereas fermentation depends on the metabolic activities of microorganisms and their enzymes to convert available substrates into organic acids, peptides, alcohols, gases, and other metabolites (Xiang *et al*., 2019; Cruz-Casas *et al*., 2021). These approaches have been applied to a wide range of food materials, including plant proteins, cereals, legumes, fruits, dairy and dairy-alternative products, microalgae, and food-processing residues, although their effects vary with substrate characteristics and processing conditions (Abdullahi *et al*., 2021; Cruz-Casas *et al*., 2021). Enzymes are also important processing aids because their catalytic activity can improve food quality, stability and sensory characteristics while supporting more efficient processing and waste reduction (Abdullahi *et al*., 2021).

The complementary nature of these processes provides an opportunity to combine targeted substrate modification with subsequent or simultaneous microbial biotransformation. Exogenous enzymes, such as proteases, cellulases, pectinases, and other carbohydrases, can hydrolyze proteins and structural polysaccharides, disrupt cell-wall components, and improve the accessibility of compounds embedded within food matrices (Cruz-Casas *et al*., 2021; Mora & Toldrá, 2023; Zhu *et al*., 2023). Proteolytic hydrolysis can decrease protein molecular size and generate peptides with modified solubility, digestibility, and functional properties, whereas carbohydrase-mediated hydrolysis can alter cell-wall polysaccharides and promote the release of phytochemicals associated with the food matrix (Murtaza *et al*., 2022; Rafique *et al*., 2022; Song *et al*., 2022). Fermentation can subsequently transform these released substrates through microbial proteolysis, acidification, enzymatic biotransformation, and the formation of secondary metabolites. Studies involving pea protein, lupin proteins, cereal substrates, and other food matrices suggest that these complementary activities may produce effects that differ from those achieved through either process independently (Schlegel *et al*., 2019; Schlegel *et al*., 2021; García-Arteaga *et al*., 2022; Xu *et al*., 2024).

Since the two processes operate through distinct but interconnected biochemical pathways, their sequence of application can strongly influence the final outcome. Applying enzymatic hydrolysis before fermentation may improve substrate accessibility and provide microorganisms with smaller molecular substrates that are more readily utilized. In contrast, fermentation before enzymatic treatment may modify protein conformation, cell-wall architecture, acidity, or substrate composition, thereby affecting subsequent hydrolysis. Experimental evidence supports this dependence on processing sequence. García-Arteaga *et al*. (2022) reported that enzymatic hydrolysis combined with *Lactobacillus plantarum* fermentation modified the characteristics of pea protein, with the processing sequence influencing the resulting properties. Similarly, research on oat yogurt showed that optimization of enzymatic hydrolysis and fermentation conditions affected acidity, coagulation, viscosity, and textural properties (Xu *et al*., 2024). Multi-stage processing of bee pollen also showed that treatment sequence influenced phenolic and flavonoid contents, antioxidant activity, and protein-related responses (Kaškonienė *et al*., 2026). These findings suggest that enzyme-assisted fermentation should be viewed not as a uniform treatment, but as a range of processing configurations whose outcomes depend on the sequence, intensity, and conditions of the individual operations.

This process dependence is particularly important in protein-rich foods and food-derived materials, where enzymatic hydrolysis and fermentation can contribute to the generation of bioactive peptides from intact proteins. Food proteins contain peptide sequences that may exhibit antioxidant, antihypertensive, antimicrobial, immunomodulatory, or enzyme-inhibitory activities following their release from parent proteins (Daliri *et al*., 2017; Chakrabarti *et al*., 2018; Akbarian *et al*., 2022). Recent reviews indicate that the properties of the resulting peptides are influenced by protein source, enzyme specificity, degree of hydrolysis, molecular-weight distribution, and peptide sequence (Cruz-Casas *et al*., 2021; Mora & Toldrá, 2023; Farias & Kempka, 2026). Fermentation may complement enzymatic hydrolysis by introducing microbial proteases and other metabolic activities that further modify peptide profiles. Studies involving pea, lupin, whey, sesame, hempseed, and other protein-rich substrates demonstrate the potential of combined or sequential processing to influence digestibility, solubility, functional properties, and measured biological activities (Schlegel *et al*., 2021; García-Arteaga *et al*., 2022; Kim *et al*., 2024; Feng *et al*., 2025; Raei *et al*., 2025).

The potential formation of bioactive peptides also requires a clear distinction between biochemical activities measured in a model system and demonstrated health effects. The presence of bioactive peptides or an increase in an in-vitro biological activity should not automatically be interpreted as evidence of physiological health benefits. Peptide activity can depend on amino-acid sequence, molecular size, structural characteristics, gastrointestinal stability, bioaccessibility and the experimental model used for evaluation (Tadesse & Emire, 2020; Akbarian *et al*., 2022; Mora & Toldrá, 2023). For example, DPPH, ABTS, FRAP, CUPRAC, ACE-inhibition and related assays generate distinct chemical or biochemical measurements and therefore should not be interpreted as equivalent indicators of health outcomes. Accordingly, findings describing antioxidant, antimicrobial, antidiabetic, or enzyme-inhibitory activities require careful interpretation, especially when the supporting evidence is limited to chemical assays or in-vitro models. The literature also highlights the need for adequate peptide characterization and assessment of bioaccessibility before such findings can be meaningfully translated into functional-food or health-related applications.

These matrix-level interactions are not limited to proteins but also involve phenolic compounds, flavonoids, carotenoids, and other phytochemicals. Enzymes that act on plant cell-wall structures can promote the release of compounds that are physically entrapped within the matrix or chemically associated with its structural components. Subsequent fermentation may further modify these compounds through microbial enzymes and metabolic processes. Thus, observed increases or reductions in phytochemical levels may reflect their release, degradation, transformation, or conversion into newly formed metabolites, rather than simple changes in concentration (Li *et al*., 2024; Aregbe *et al*., 2025; Kaškonienė *et al*., 2026). In bee pollen, multi-stage enzymatic and microbial processing produced changes in phenolic and flavonoid contents and antioxidant responses (Kaškonienė *et al*., 2026), while enzyme-assisted fermentation of sea-buckthorn cereal beverages altered phytochemical composition and antioxidant-related properties (Aregbe *et al*., 2025). Similarly, the combined application of enzymatic hydrolysis and fermentation to whole citrus materials resulted in measurable changes in phenolic and flavonoid contents, carotenoid levels, and antioxidant activity (Li *et al*., 2024). Enzymatic pretreatment followed by lactic fermentation of *Chlorella vulgaris* also produced changes in phenolic content and antioxidant responses measured using CUPRAC, ABTS and DPPH assays (Bilgin *et al*., 2026).

Beyond changes in phytochemical composition, these transformations may also affect nutritional accessibility through their influence on antinutritional factors and other matrix components. Fermentation and enzymatic treatment can modify phytate, oligosaccharides, protein accessibility, and other food components that influence nutrient utilization. For example, processing of a fermented lentil beverage was associated with reductions in selected antinutritional components, including phytate and raffinose-family oligosaccharides, alongside changes in protein-related characteristics (Verni *et al*., 2020). Research on cereal and plant-protein systems has likewise shown that hydrolysis and fermentation can modify molecular structures and influence the accessibility of nutrients and functional constituents (Schlegel *et al*., 2019; Chen *et al*., 2022; García-Arteaga *et al*., 2022). However, a reduction in the concentration of an individual antinutritional compound should not, by itself, be interpreted as evidence of enhanced mineral or nutrient bioavailability unless bioavailability has been directly determined.

Nutritional and phytochemical changes also occur within the food matrix and may influence its technological performance. In addition to altering nutritional composition, enzyme-assisted fermentation can affect the functional, physicochemical, and sensory properties of food products. Enzymatic hydrolysis may modify molecular-weight distribution, solubility, water-holding capacity, emulsifying and foaming properties, whereas fermentation can influence acidity, protein interactions, starch structure, and the formation of microbial metabolites (Schlegel *et al*., 2019; Schlegel *et al*., 2020; Xu *et al*., 2024). In brown rice, cellulase treatment followed by *Lactobacillus plantarum* fermentation reduced hardness and increased stickiness, indicating that combined biological processing can substantially influence texture (Lu *et al*., 2025). Similarly, optimization of enzymatic hydrolysis and fermentation in oat yogurt affected acidity, coagulation, viscosity, and cohesiveness (Xu *et al*., 2024). These observations indicate that nutritional improvement

should not be considered separately from technological functionality, since structural changes within the matrix may simultaneously influence processing behavior and attributes relevant to consumer acceptance.

The effects of these biochemical and structural transformations can also extend to flavour and aroma development. Microbial metabolism can produce organic acids, alcohols, esters, aldehydes, and other volatile compounds, while enzymatic hydrolysis may release precursors that undergo subsequent microbial or chemical transformations. In butter, enzymatic hydrolysis followed by *Lactococcus lactis* fermentation altered the volatile profile and contributed to the development of cheese-like flavour characteristics (Fan *et al*., 2025). Similarly, enzyme-assisted co-fermentation of chili paste modified volatile compounds and aroma-related properties (Liu *et al*., 2026). These findings demonstrate the potential of biological processing to influence not only nutritional characteristics but also sensory attributes. Nevertheless, excessive hydrolysis or poorly controlled fermentation may result in undesirable bitterness, off-flavours, or adverse textural changes. Process optimization and sensory assessment are therefore essential during product development.

The same biochemical principles also provide opportunities for valorization of food-processing by-products and other underutilized biomass. Agro-industrial residues, including wheat bran, brewers' spent grain, spent coffee grounds, oil-extraction residues, and kitchen food waste, contain proteins, polysaccharides, minerals, and phenolic compounds that can potentially be recovered or transformed through biological processing. Fermentation-based valorization of cereal wastes has been proposed as a means of converting low-value residues into higher-value ingredients and bioproducts (Adil *et al*., 2026). Enzymatic pretreatment combined with fermentation has similarly been investigated for brewers' spent grain to improve substrate conversion and modify phenolic and carbohydrate fractions (Moirangthem *et al*., 2024). Sequential pretreatment, enzymatic hydrolysis, and selective fermentation have also been explored for spent coffee grounds, while integrated enzymatic hydrolysis and fermentation have been investigated for the valorization of mixed kitchen food waste (Cho *et al*., 2025; Arvelli *et al*., 2026). These approaches link enzyme-assisted fermentation with circular-bioeconomy principles by seeking to recover valuable constituents and generate useful products from materials that might otherwise be discarded.

Against this background, the present systematic review synthesizes evidence on enzymatic hydrolysis and microbial fermentation across food and food-related matrices, with particular emphasis on their combined application. The review examines how substrate characteristics, enzyme systems, microbial strains, processing sequence, and operating conditions influence nutritional, functional, bioactive, physicochemical, and sensory outcomes, including protein digestibility, phytochemical transformation, antinutritional factors, antioxidant-related activities, and by-product valorization.

The review therefore considers integrated enzymatic-microbial processing a context-dependent strategy rather than a universally beneficial treatment. It distinguishes direct evidence from studies of combined processing from contextual evidence used to clarify mechanisms, applications, safety, and scale-up, while identifying methodological and technological gaps that may affect reproducibility and practical implementation. This framework provides the basis for the thematic synthesis presented in the subsequent sections.

Literature Search Strategy and Eligibility Criteria

The literature search and evidence synthesis were conducted iteratively and concurrently from September to November 2026. The search process was designed to identify, assess, and synthesize studies addressing enzymatic hydrolysis, enzyme-assisted fermentation, enzymatic treatment, microbial fermentation, and their applications in food and food-related matrices. Searches were conducted in Web of Science and PubMed and were supplemented by searches using Google Scholar and Google. Targeted searches were also carried out on the publisher platforms Taylor & Francis Online, ScienceDirect (Elsevier), and SpringerLink. The iterative approach allowed relevant publications to be identified as the thematic synthesis developed, while emerging findings and areas requiring further clarification informed subsequent searches and the examination of additional literature.

The search strategy was developed around three major concept groups: processing interventions, food materials, and relevant research outcomes. Processing-related terms included enzymatic hydrolysis, enzyme-assisted fermentation, enzymatic treatment, enzyme treatment, fermentation, and microbial fermentation. Food-related terms included food, food processing, food matrix, cereals, legumes, plant-based foods, food by-products, and agro-industrial residues. Outcome-related terms comprised nutritional quality, functional properties, techno-functional properties, bioactive compounds, antioxidant activity, bioavailability, sustainability, circular economy, and waste valorisation. Boolean operators were used to combine these terms systematically, with OR connecting related terms within each concept group AND linking the different concept groups. Broad search combinations were used to identify studies investigating enzymatic treatments, microbial fermentation, or their combined applications in food systems, while more focused combinations incorporating both enzyme-related and fermentation-related terms were used to identify studies specifically examining integrated processing approaches. Additional outcome-related terms were incorporated into targeted searches to capture relevant evidence on nutritional, functional, bioactive, technological, and sustainability-related effects. Search terms and their combinations were adjusted where necessary to accommodate the search functionality of the different databases and publisher platforms.

The eligibility criteria encompassed peer-reviewed publications written in English that addressed enzymatic hydrolysis, microbial fermentation, or the combined application of both approaches in food and food-compatible materials. Eligible publications were classified into three broad categories. The first comprised primary experimental studies investigating enzymatic treatments, microbial fermentation, or integrated enzymatic-microbial processing and reporting relevant nutritional, functional, bioactive, physicochemical, sensory, safety, or sustainability-related outcomes. The second category included review articles and other secondary publications that provided information on advances, applications, mechanisms, and emerging developments in enzyme-based processing and microbial fermentation. The third category consisted of contextual and mechanistic studies addressing enzyme systems, microbial activities, biochemical transformations, processing conditions, and the valorisation of food by-products, where such information contributed to the interpretation of the review findings. Publications were excluded if they were duplicates, written in languages other than English, unrelated to food or food-compatible applications, or lacked sufficient

methodological or outcome information relevant to the objectives of the review. The review primarily covered literature published between 2017 and 2026. One older reference published in 2011 was retained as background literature on the enzymatic hydrolysis of soy proteins and the potential applications of the resulting hydrolysates.

The literature search initially identified 82 records. Following the removal of nine duplicate records, 73 unique records remained for eligibility screening. Among these, four were excluded because they did not meet the predefined inclusion criteria, leaving 69 publications for the original evidence synthesis. These comprised 17 primary experimental studies that directly investigated the combined application of enzymatic and microbial processing in food materials or food-compatible ingredients, forming the core evidence base for comparative analysis. The remaining 52 publications provided complementary evidence on individual processing approaches, biochemical mechanisms, broader food applications, safety considerations, and technological scale-up.

In addition to these 69 publications, four supplementary contextual publications were incorporated during the iterative synthesis to strengthen specific aspects of the discussion. These publications provided additional background on enzyme-based processing, microbial fermentation, functional food development, and emerging biotechnological applications. Although they contributed to the review's discussion and reference list, they were not part of the original 82 record screening process and were not considered direct evidence for the comparative evaluation of integrated enzymatic-microbial processing. Consequently, the review drew on a total of 73 publications, comprising 69 publications retained through the original screening process and four supplementary contextual publications. Figure 1 presents the literature search and selection process, showing the progression from the initial 82 records through duplicate removal, eligibility screening, exclusion of four records, and the subsequent incorporation of four contextual publications during the iterative synthesis.



Figure 1: Flow diagram of the literature search and selection process

Conceptual Framework

The conceptual framework was developed to organize the relationships among food substrates, enzymatic hydrolysis, microbial fermentation, processing sequence, biochemical transformations, and food-quality outcomes identified across the reviewed literature. Figure 2 presents the conceptual framework used to guide the interpretation and thematic synthesis of the evidence.

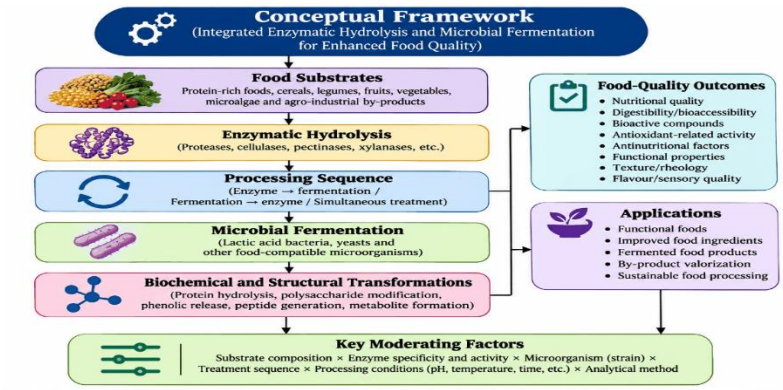


Figure 2: Conceptual framework for integrated enzymatic hydrolysis and microbial fermentation

The framework begins with the different food substrates and food-derived materials represented in the literature, including protein-rich foods, cereals, legumes, fruits, vegetables, microalgae, and agro-industrial by-products. Differences in their structural and compositional characteristics can influence the accessibility of proteins, polysaccharides, phenolic compounds, and other bioactive constituents during processing.

The first major processing component is enzymatic hydrolysis, with particular emphasis on proteolytic and carbohydrase-based treatments. Proteases promote protein hydrolysis and peptide formation, whereas cellulases, pectinases, hemicellulases, xylanases, and related enzymes act on structural polysaccharides and plant cell-wall components. These reactions may improve substrate accessibility, facilitate the release of matrix-associated compounds, and generate smaller or more readily fermentable substrates for subsequent microbial activity. The magnitude of these effects depends on enzyme specificity and activity, pH, temperature, treatment duration, and the structural and compositional characteristics of the food matrix.

The framework then incorporates different processing configurations, including enzymatic hydrolysis followed by fermentation, fermentation followed by enzymatic hydrolysis, and simultaneous treatment. These configurations are not assumed to produce equivalent effects. Their outcomes may vary according to substrate composition, enzyme characteristics, microbial strain, and processing conditions. Consequently, treatment sequence represents an important factor when comparing results across different food systems.

The next component is microbial fermentation, involving lactic acid bacteria, yeasts, and other food-compatible microorganisms. Through microbial metabolism and associated enzymatic activities, fermentation can transform compounds naturally present in the substrate as well as those released during preceding enzymatic treatment. The interaction between enzymatic hydrolysis and microbial fermentation can consequently influence substrate availability, microbial growth, metabolite production, and the physicochemical and functional characteristics of the resulting food products.

These processing stages may lead to a range of biochemical and structural transformations, including protein hydrolysis, polysaccharide modification, release or transformation of phenolic compounds, generation of peptides, and formation of microbial metabolites. These transformations provide a mechanistic link between processing interventions and the food-quality outcomes reported across the literature.

The resulting food-quality outcomes include nutritional quality, protein digestibility and bioaccessibility, bioactive compounds, antioxidant-related activity, antinutritional factors, functional and physicochemical properties, texture and rheological characteristics, and flavour and sensory quality. The framework also recognizes the potential application of these processes in functional food development, improved food ingredients, fermented food products, by-product valorisation, and sustainable food processing.

Finally, the framework recognizes several key moderating factors that can influence processing effectiveness. These include substrate composition, enzyme specificity and activity, microbial strain, treatment sequence, pH, temperature, treatment duration, analytical methods, process standardisation, safety and regulatory considerations, scalability, and economic feasibility. These factors are important when interpreting differences among studies and when considering the translation of laboratory-scale findings into practical food-processing applications.

Overall, the conceptual framework provides a structured basis for interpreting the reviewed evidence. It connects food substrates and processing interventions with biochemical and structural transformations, food-quality outcomes, and potential food and sustainability applications, while recognizing that the effectiveness of integrated enzymatic-microbial processing is strongly dependent on the characteristics of the substrate, processing configuration, biological agents, operating conditions, and analytical approach.

RESULTS AND DISCUSSION

Evidence Characteristics and Process Diversity

The 73 publications retained through the original screening process covered a diverse range of food and food-compatible matrices, including plant proteins, pulses, cereals, fruits, dairy and dairy-alternative products, bee pollen, microalgae, fermented condiments, wheat bran, brewers' spent grain, oil-extraction residues, and other underutilized materials. The core studies examining combined processing were concentrated mainly on plant proteins, cereal-based beverages, fermented beverages, and other plant-derived matrices. The four supplementary contextual publications broadened the discussion of food-enzyme applications, fermentation-based food development, and emerging biotechnological approaches. Overall, this distribution suggests that integrated enzymatic-microbial processing is more appropriately viewed as a group of matrix-specific approaches rather than as a single standardized technology.

Studies involving protein-based systems generally focused on proteolysis, reduction in molecular weight, solubility, foaming and emulsifying properties, digestibility, and peptide bioactivity. In cell-wall-rich matrices, greater attention was given to the release of phenolic compounds and flavonoids, production of fermentable carbohydrates, antioxidant responses, and textural changes. Studies involving fermented dairy and dairy-alternative systems additionally examined acidity, gel formation, viscosity, aroma, and volatile compounds. The range of outcomes reflects the multifaceted effects of fermentation reported in the wider literature, where microbial metabolism can contribute to nutritional, sensory, and functional changes (Xiang *et al*., 2019; Fitsum *et al*., 2025; Künili *et al*., 2025).

Integrated Enzymatic Hydrolysis and Microbial Fermentation

The core studies identified in the evidence base indicate that combining enzymatic and microbial processing can influence protein structure, phytochemical composition, antioxidant-related activity, texture, flavour, and other functional properties. However, the magnitude and direction of these effects varied according to the substrate, enzyme system, microorganism, treatment sequence, and processing conditions. This variability provides a useful basis for first considering the influence of treatment sequence and subsequently examining the major nutritional, phytochemical, structural, sensory, and valorisation outcomes reported across the different food systems (García-Arteaga *et al*., 2022; Li *et al*., 2024; Bilgin *et al*., 2026; Kaškonienė *et al*., 2026).

Influence of Treatment Sequence

Treatment sequence was an important factor in several of the identified studies. In pea protein isolate, García-Arteaga *et al*. (2022) compared different combinations of enzymatic

hydrolysis and *Lactobacillus plantarum* fermentation and found that treatment order affected protein molecular characteristics, solubility, foaming properties, antigenic proteins and sensory characteristics. The study reported particularly pronounced changes when fermentation was followed by enzymatic hydrolysis, indicating that the two operations were not interchangeable (García-Arteaga *et al*., 2022).

A sequence-dependent response was also examined in bee pollen. Kaškonienė *et al*. (2026) compared the two possible sequences of multi-stage enzymatic and bacterial treatment. Although the abstract reported substantial increases following enzyme-first processing, the detailed results indicated that the response varied according to the pollen sample and enzyme preparation. Clara-diastase combined with bacterial fermentation frequently produced the greatest increases, regardless of the treatment sequence. Thus, the study supports the use of multi-stage processing but does not establish enzyme-first treatment as a universally superior sequence. Increases were observed in total phenolic content, total flavonoid content, radical-scavenging activity, and inhibition of heat-induced BSA denaturation (Kaškonienė *et al*., 2026).

Similarly, Xu *et al*. (2024) used enzymatic liquefaction and saccharification before fermentation during the development of set-type oat yogurt. The optimized treatment resulted in favorable acidity, viscosity, cohesiveness, and coagulation characteristics. Exopolysaccharide production also contributed to the structural properties of the fermented product (Xu *et al*., 2024).

Taken together, these findings indicate that treatment sequence is an important factor influencing substrate accessibility and subsequent biochemical transformations, although no single sequence appears to be consistently optimal across different food matrices. This sequence dependence is particularly relevant when considering protein transformation and functionality, where the initial molecular structure of the protein and the extent of hydrolysis can strongly influence the resulting properties.

Protein Transformation, Digestibility and Functionality

Protein-rich substrates constituted an important component of the core evidence. Because treatment sequence determines the biochemical conditions under which hydrolysis and microbial metabolism occur, changes in protein molecular characteristics and functional properties varied according to the protein source and processing conditions (Verni *et al*., 2020; Schlegel *et al*., 2021; García-Arteaga *et al*., 2022). A formulation-level example was reported by Dridi *et al*. (2021), who enzymatically treated cricket protein before incorporating it into a commercial probiotic-fermented beverage. Since the beverage matrix had already undergone fermentation, this study does not provide direct evidence for an enzyme-first fermentation sequence applied to the cricket protein itself. Rather, it demonstrates the use of an enzyme-modified ingredient within a fermented food matrix.

In pea protein isolate, García-Arteaga *et al*. (2022) reported improvements in protein solubility and foaming capacity following combined enzymatic and lactic fermentation treatments. Changes in molecular-weight distribution and immunological detection were also observed, while sensory evaluation indicated a reduction in characteristic pea off-flavours in some treatments. These findings demonstrate that combined processing can influence protein structure, technological functionality, and sensory characteristics simultaneously (García-Arteaga *et al*., 2022).

Comparable effects were observed in lupin protein. Schlegel *et al*. (2021) combined enzymatic hydrolysis using papain, Alcalase and pepsin with lactic acid fermentation. The fermented hydrolysates showed improved solubility and foaming activity compared with untreated lupin protein isolate. The study also reported a marked reduction in the detectable lupin allergen Lup an 1 following combined treatment (Schlegel *et al*., 2021).

Similarly, Verni *et al*. (2020) reported changes in a fermented lentil beverage following proteolytic treatment and subsequent fermentation with *Lactobacillus*. The process generated soluble proteins, peptides and amino acids and reduced selected antinutritional components, including phytic acid and raffinose-family oligosaccharides (Verni *et al*., 2020).

Taken together, these studies suggest that enzymatic-microbial processing can modify protein accessibility and functional properties while also influencing selected antinutritional and allergenic components. These protein-level effects provide a basis for examining the release and subsequent transformation of phytochemicals in plant-based matrices. Nevertheless, the available evidence does not indicate that combined processing produces uniform nutritional or functional outcomes across all food systems, since enzyme specificity, degree of hydrolysis, microbial strain, and treatment sequence vary considerably among studies (Verni *et al*., 2020; Schlegel *et al*., 2021; García-Arteaga *et al*., 2022).

Phytochemical Release, Transformation and Antioxidant-Related Responses

Plant-based matrices provided substantial evidence for the effects of combined processing on phenolic compounds, flavonoids and antioxidant-related responses. These effects were particularly evident in substrates in which enzymatic treatment could facilitate the release of compounds associated with the food matrix (Chen *et al*., 2022; Li *et al*., 2024; Aregbe *et al*., 2025; Bilgin *et al*., 2026).

In sea-buckthorn cereal beverages, Aregbe *et al*. (2025) reported increases in total phenolics, flavonols, flavonoids and proanthocyanidins following enzyme-assisted fermentation. The study also observed changes in antioxidant-related and enzyme-inhibition assays and reported modifications in the volatile profile of the beverages (Aregbe *et al*., 2025).

Li *et al*. (2024) investigated whole red mandarin using pectinase, cellulase and hemicellulase treatment followed by lactic acid fermentation. Enzymatic treatment facilitated the release of compounds from the fruit matrix, while subsequent fermentation was associated with additional changes in phenolic and flavonoid contents. The authors related some of these changes to microbial enzymatic activities involved in the transformation of phenolic compounds and flavonoid glycosides.

Bilgin *et al*. (2026) evaluated enzymatic pretreatment followed by lactic acid fermentation of *Chlorella vulgaris*. The combined treatment resulted in measurable total phenolic content and antioxidant activity across the CUPRAC, ABTS, and DPPH assays. However, the authors reported that most of the differences between the fermented and enzyme-treated samples were not statistically significant. The findings therefore support the potential of the combined process but do not justify treating every measured increase as a statistically distinct improvement over the individual treatments.

The coarse cereal compound powder study also reported changes in antioxidant-related properties, anthocyanins, flavones and soluble dietary fiber following enzymolysis, fermentation and their combination (Chen *et al*., 2022). These findings collectively indicate that enzymatic and microbial

processing can facilitate the release or transformation of matrix-associated phytochemicals, although the extent of the response varies among food matrices and processing conditions (Chen *et al*., 2022; Li *et al*., 2024; Aregbe *et al*., 2025).

The antioxidant findings require careful interpretation because the studies used different analytical methods, including DPPH, ABTS, FRAP and CUPRAC (Chen *et al*., 2022; Aregbe *et al*., 2025; Bilgin *et al*., 2026). These assays measure different chemical responses and should therefore not be treated as interchangeable indicators. Accordingly, an increase observed in one antioxidant assay should be interpreted within the context of the individual study rather than assumed to represent a comparable increase in physiological antioxidant activity. Having established this analytical limitation, the synthesis now considers the physical and technological consequences of the biochemical transformations associated with combined processing.

Structural Modification and Technological Functionality

Combined enzymatic and microbial processing also resulted in changes in texture, viscosity, water interactions, starch characteristics, and other technological properties. Such responses have been reported in cereal- and plant-based matrices subjected to enzymatic treatment followed by fermentation (Chen *et al*., 2022; Xu *et al*., 2024; Lu *et al*., 2025).

In oat yogurt, Xu *et al*. (2024) demonstrated that enzymatic liquefaction and saccharification before fermentation affected acidity, viscosity, cohesiveness and coagulation. The optimized process produced a coagulated product with favorable rheological characteristics, while microbial exopolysaccharide production contributed to the final texture.

Lu *et al*. (2025) reported a distinct structural response in whole brown rice following treatment with cellulase and *Lactiplantibacillus plantarum* P-8. Compared with the individual treatments, the combined process reduced hardness and increased stickiness. Changes were also observed in water absorption and starch characteristics, indicating modification of the cereal matrix by the combined treatment.

Chen *et al*. (2022) similarly reported changes in gelatinization behaviour, particle size, and microstructure following the enzymolysis and fermentation of a coarse cereal compound powder. The combined treatment produced structural characteristics that differed from those observed following enzymolysis alone.

Overall, the core evidence indicates that combined enzymatic and microbial processing can modify technological functionality through changes in protein structure, polysaccharide degradation, starch behaviour, matrix integrity, and the production of microbial metabolites (Chen *et al*., 2022; Xu *et al*., 2024; Lu *et al*., 2025). These structural and biochemical changes are closely related to consumer-relevant attributes, particularly flavour and aroma, which are considered in the next outcome domain.

Sensory and Flavour Development

Sensory and flavour characteristics were also influenced by combined enzymatic and microbial processing. Enzymatic hydrolysis can release peptides, amino acids, and soluble carbohydrates, while microbial fermentation generates metabolites that contribute to the development of aroma and flavour (Fan *et al*., 2025; Liu *et al*., 2026).

In fermented chili paste, Liu *et al*. (2026) investigated LAB-yeast co-fermentation with and without cellulase and Flavorzyme. The enzyme-assisted co-fermentation altered acidity, amino-acid nitrogen, residual sugar levels, capsaicinoids, and volatile compounds. The study identified 166 volatile compounds and reported changes in several groups of flavor-associated metabolites. Sensory evaluation also indicated differences among the processing treatments.

Fan *et al*. (2025) investigated enzymatic hydrolysis combined with *Lactococcus lactis* fermentation of butter. GC–MS analysis identified 218 volatile metabolites, including esters, acids and ketones. The authors associated the development of cheese-like flavor characteristics with interactions between enzymatic hydrolysis and microbial fermentation.

The millet-yogurt study also reported changes in volatile and non-volatile metabolites following processing with an enzymatic fermentation broth and *Lactiplantibacillus plantarum*. Increased SOD activity and changes in flavour-associated compounds were also reported, together with a reduction in some undesirable volatile characteristics (Fan *et al*., 2022).

These findings extend the preceding nutritional and functional evidence by showing that integrated processing can also influence flavour development and sensory quality. However, the sensory effects are not necessarily uniformly positive. For example, Liu *et al*. (2026) reported that enzyme treatment influenced texture while also improving some aroma- and taste-related characteristics. Therefore, process optimization should consider nutritional, technological, and sensory responses simultaneously rather than treating sensory quality as a secondary outcome (Fan *et al*., 2025; Liu *et al*., 2026). These product-level considerations lead naturally to a broader application of the technology: valorization of food-processing by-products, where process performance must be balanced against food quality and practical feasibility.

By-Product Valorization and Resource Recovery

The evidence also supports the application of enzyme-assisted fermentation to food-processing by-products, thereby connecting bioprocessing with food-resource valorization. Brewers' spent grain and wheat bran are representative examples in which enzymatic treatment has been used to improve substrate accessibility before or during microbial fermentation (Moirangthem *et al*., 2024; Hassan *et al*., 2025).

Moirangthem *et al*. (2024) investigated brewers' spent grain using a commercial multi-enzyme preparation containing cellulase, xylanase, α-amylase, protease, lipase and pullulanase activities, together with *Lactiplantibacillus plantarum*. The untreated substrate supported limited microbial growth because of restricted availability of fermentable substrates. In contrast, the combined enzymatic and microbial treatment enhanced acidification and lactic acid production while modifying the phenolic acid profile, including the release of ferulic acid.

The evidence from wheat bran similarly demonstrates the potential of pretreatment–fermentation combinations. Hassan *et al*. (2025) applied xylanase pretreatment followed by fermentation with L. plantarum and *Saccharomyces cerevisiae*. The treatment increased water-extractable arabinoxylans and phenolic compounds, reduced phytic acid, and improved selected technological characteristics.

Taken together, these findings demonstrate the potential of combined bioprocessing to transform fibre-rich and otherwise underutilized food-processing materials into ingredients with modified nutritional and technological properties (Moirangthem *et al*., 2024; Hassan *et al*., 2025). However, translating these laboratory-scale findings into practical food applications requires further consideration of sensory acceptability, process reproducibility, safety, economic feasibility, and scale-up conditions (Moirangthem *et al*.,

2024). These considerations provide an important basis for interpreting the broader evidence regarding reproducibility, safety, standardization, and industrial scale-up.

Overall Interpretation

The reviewed studies demonstrate that combined enzymatic and microbial processing can influence multiple dimensions of food quality, including molecular composition, nutritional characteristics, functional properties, bioactive compounds, antioxidant-related responses, and sensory attributes (García-Arteaga *et al*., 2022; Li *et al*., 2024; Lu *et al*., 2025; Kaškonienė *et al*., 2026; Liu *et al*., 2026).

However, the available literature does not establish that combined treatment is universally superior to enzyme-only or fermentation-only processing. The magnitude and direction of the observed effects varied according to the food matrix, enzyme system, microbial strain, treatment sequence, processing conditions, and outcome measured. In some systems, integration of the two approaches produced complementary or enhanced effects, whereas in others, the additional treatment did not result in statistically significant improvements over individual processing approaches. These findings support the interpretation of enzymatic–microbial processing as a context-dependent strategy whose effectiveness depends on matching the processing configuration to the characteristics and intended use of the target food matrix. In *Chlorella vulgaris*, for example, combined processing produced changes in phenolic and antioxidant measurements, but several differences between treatment groups were not statistically significant (Bilgin *et al*., 2026). In whole brown rice, the combined treatment produced pronounced changes in hardness, stickiness and water-related properties, but the magnitude of the response differed among the individual structural parameters (Lu *et al*., 2025). Such findings emphasize the importance of comparing combined treatments with appropriate enzyme-only and fermentation-only controls.

Overall, the evidence indicates that the effectiveness of enzyme-assisted fermentation is influenced by substrate composition, enzyme specificity, microbial strain, treatment sequence, and processing conditions (García Arteaga *et al*., 2022; Moirangthem *et al*., 2024; Kaškonienė *et al*., 2026). Studies that included appropriate controls and compared alternative treatment sequences provided stronger evidence for distinguishing the effects attributable to enzymatic hydrolysis from those associated with microbial fermentation.

Taken together, the core evidence supports the view that enzyme-assisted fermentation is a flexible, matrix-dependent bioprocessing strategy rather than a single standardized technology. Its applications extend from protein modification and phytochemical release to changes in texture, flavour development, and the valorisation of food-processing by-products (Chen *et al*., 2022; Li *et al*., 2024; Moirangthem *et al*., 2024; Fan *et al*., 2025; Hassan *et al*., 2025).

The principal findings from the core combined-processing studies are summarized in Table 1. Having established the major patterns emerging from these studies, the subsequent sections broaden the synthesis to the wider evidence base, beginning with protein hydrolysis, digestibility, and bioactive peptide formation.

Table 1: Primary Studies Evaluating Integrated Enzymatic–Microbial Processing and Formulation-Level Combinations

Study / Food Matrix	Treatment and Configuration	Principal Outcomes and Interpretation
Bee pollen	Enzymatic hydrolysis + LAB fermentation Configuration: Both treatment orders tested; responses depended on pollen sample and enzyme preparation	Phenolics, flavonoids, antioxidant/biological assays. Multi-stage treatment increased measured phenolics, flavonoids and in-vitro activity; detailed results did not establish a universal order advantage (Kaškonienė *et al*., 2026).
Sea-buckthorn cereal beverage	Enzyme-assisted fermentation Configuration: Enzyme-assisted fermentation	TPC, TFC, flavonols, proanthocyanidins, antioxidant, ACE-inhibitory and antidiabetic assays. Phytochemical and in-vitro bioactivity responses increased (Aregbe *et al*., 2025).
Pea protein isolate	Proteases + L. plantarum Configuration: Different sequences	Antigenic proteins, solubility, foaming, sensory. Treatment sequence altered molecular and functional responses (García Arteaga *et al*., 2022).
Oat yogurt	Enzymatic hydrolysis + yogurt fermentation Configuration: Hydrolysis → fermentation	Acidity, texture, viscosity, EPS. Optimization produced favorable acidity and coagulated texture (Xu *et al*., 2024).
Millet yogurt	Enzymatic treatment + fermentation Configuration: Millet enzymatic digestion → *Bacillus subtilis* fermentation; broth incorporated into LAB-fermented yogurt	SOD, antioxidant capacity, sensory. The millet-derived enzymatic-fermentation broth contributed SOD-related and flavour/metabolite characteristics to the yogurt formulation (Fan *et al*., 2022).
Lupin protein hydrolysates	Protease hydrolysis + LAB fermentation Configuration: Hydrolysis → fermentation	Solubility, foaming, allergen protein, sensory. Fermentation modified functionality and allergenic-protein response (Schlegel *et al*., 2021).
Coarse cereal compound powder	Enzymolysis + fermentation Configuration: Individual and joint treatment	DPPH, ABTS, radical scavenging, chelation, anthocyanins/flavones, dietary fibre. Joint processing altered antioxidant and functional profiles (Chen *et al*., 2022).
Cricket-protein beverage	Enzymatic pretreatment + fermentation Configuration: Enzyme-treated cricket protein incorporated into an already probiotic-fermented beverage	Digestibility, peptide profile, nutritional/functional properties. Formulation-level integration; the study did not test fermentation of the enzyme-treated cricket

Study / Food Matrix	Treatment and Configuration	Principal Outcomes and Interpretation
		protein or compare enzyme→fermentation sequence (Dridi *et al*., 2021).
Fermented chili paste	Cellulase/flavourzyme + LAB/yeast Configuration: Co-fermentation	Volatiles, amino-acid nitrogen, capsaicinoids, sensory. Enzymatic hydrolysis altered volatile and flavour development (Liu *et al*., 2026).
Lentil beverage	Proteolytic treatment + Lactobacillus Configuration: Hydrolysis → fermentation	Soluble protein, digestibility, phytate, raffinose. Selected antinutritional factors decreased while protein accessibility improved (Verni *et al*., 2020).
Pea protein	Enzymatic treatment + LAB Configuration: Enzyme → fermentation	Digestibility, bioavailability, molecular weight. Reported higher early-stage in-vitro digestibility and Caco-2 bioavailability (Kim *et al*., 2024).
Butter	Enzymatic hydrolysis + L. lactis Configuration: Hydrolysis → fermentation	Volatiles, flavour. Cheese-like volatile and flavour profiles were developed (Fan *et al*., 2025).
Whole red mandarin	Cellulase/pectinase/hemicellulase + LAB Configuration: Hydrolysis → fermentation	Nutrients, phytochemicals, antioxidant activity. Combined processing enhanced several measured phytochemical and antioxidant responses (Li, Y. *et al*., 2024).
Chlorella vulgaris	Enzymatic pretreatment + LAB Configuration: Pretreatment → fermentation	Phenolics, CUPRAC, ABTS, DPPH. Combined treatment produced measured phenolic and antioxidant responses; most differences between fermented and enzyme-treated samples were not statistically significant (Bilgin *et al*., 2026).
Whole brown rice	Cellulase + L. plantarum P-8 Configuration: Combined treatment	Texture, starch structure, water absorption. Hardness decreased and stickiness increased relative to controls (Lu *et al*., 2025).
Wheat bran	Xylanase + L. plantarum/yeast Configuration: Enzyme + solid-state fermentation	Phenolics, arabinoxylans, phytic acid, rheology. Combined bioprocessing improved selected nutritional and functional properties (Hassan *et al*., 2025).
Brewers' spent grain	Enzymatic pretreatment + L. plantarum Configuration: Enzyme + fermentation	Carbohydrates, phenolics, lactic acid. Pretreatment improved fermentability and changed phenolic profiles (Moirangthem *et al*., 2024).

Protein Hydrolysis, Digestibility and Bioactive Peptides

Protein-rich matrices provided some of the most direct evidence of combined effects between enzymatic and microbial processing. In pea protein isolate, García-Arteaga *et al*. (2022) reported that enzymatic hydrolysis and fermentation influenced antigenic proteins, solubility, foaming properties, and sensory characteristics, with the processing sequence also affecting the observed responses. Kim *et al*. (2024) found that enzyme-modified and fermented pea protein exhibited 22.50% greater digestion than isolated pea protein during the first 15 min of an *in vitro* digestion model and showed 38.40% higher bioavailability in a Caco-2 monolayer model. The mean molecular weight of the processed protein fraction was also substantially reduced. These findings suggest that controlled hydrolysis may improve the accessibility of protein substrates to digestive enzymes; however, the evidence remains based on laboratory and cell-based models and should not be interpreted as evidence of clinical benefit.

Bioactive peptide generation is a recurring mechanistic theme throughout the available literature. Reviews by Daliri *et al*. (2017), Chakrabarti *et al*. (2018), Cruz-Casas *et al*. (2021), Mora and Toldrá (2023), and Bruce *et al*. (2025) identify enzymatic hydrolysis and fermentation as important pathways for releasing peptide sequences from parent proteins. Such processing can alter peptide profiles through changes in protein structure, enzyme specificity, degree of hydrolysis, and microbial proteolytic activity, providing a mechanistic basis for the reported changes in digestibility and measured biological activities. The reviewed studies also illustrate substrate-specific responses: fermentation of lupin hydrolysates modified functional and allergenic characteristics (Schlegel *et al*., 2021), sea-buckthorn seed meal fermentation generated low-molecular-weight peptides with in-vitro hypoglycaemic activity (Yang *et al*., 2024), and sesame meal protein hydrolysates produced by *Bacillus coagulans* showed antioxidant and antibacterial activities (Raei *et al*., 2025).

However, greater hydrolysis is not synonymous with a better functional food. Extensive proteolysis may increase bitterness because hydrophobic peptide fragments can accumulate, a limitation observed in lupin protein studies (Schlegel *et al*., 2019; Schlegel *et al*., 2020). Therefore, process optimization should consider the degree of hydrolysis alongside peptide size distribution, sensory acceptability, and the intended functional properties of the final product. These considerations lead naturally to the next outcome domain, since changes in hydrolysis and matrix structure may also influence the release and subsequent transformation of non-protein bioactive compounds. Recent approaches have increasingly incorporated immobilized enzymes, continuous reactor systems, peptide identification, and computational tools to improve process selectivity, reproducibility, and scalability (Mora & Toldrá, 2023; Santos-Sánchez *et al*., 2024).

Phenolics, Flavonoids and Antioxidant Activity

The second major outcome domain concerned the release and transformation of phytochemicals. Bee pollen provides a useful example because its relatively resistant cell-wall structure can restrict the accessibility of phenolic compounds. Kaškonienė *et al*. (2026) reported that multi-stage processing involving enzymatic hydrolysis and bacterial fermentation increased total phenolic and flavonoid contents, together with

related activity measures. However, the magnitude of these responses varied with the pollen sample and enzyme preparation, and the detailed results did not demonstrate a universally superior treatment sequence. Aregbe *et al*. (2025) similarly observed increases in total phenolics, flavonols, total flavonoids, and proanthocyanidins during enzyme-assisted fermentation of a sea-buckthorn cereal beverage.

Whole-fruit citrus processing provided another example of the potential of integrated treatment to influence phytochemical composition. In such matrices, enzymatic disruption of cell-wall components can increase the accessibility of compounds that are otherwise retained within the plant tissue, while subsequent microbial activity may further transform the released compounds. Li *et al*. (2024) reported that combined enzymatic fermentation increased total phenols, total flavonoids, carotenoids and antioxidant activity relative to enzymatic hydrolysis alone by approximately 1.39, 1.49, 1.21 and 3.79 fold, respectively. In *Chlorella vulgaris*, enzymatic pretreatment followed by lactic fermentation produced measurable total phenolic content and responses in the CUPRAC, ABTS, and DPPH assays, although most differences between the fermented and enzyme-treated samples were not statistically significant (Bilgin *et al*., 2026). Evidence from fermentation-only studies in faba bean and table olive further indicates that microbial metabolism can modify the profile and distribution of phenolic compounds. In table olive, for example, *Lactiplantibacillus plantarum* was reported to participate in the bioconversion of oleuropein into lower-molecular-weight phenolic compounds, including hydroxytyrosol (Vaccalluzzo *et al*., 2022). Related evidence from Nigerian food-processing research also shows that microbial fermentation can modify measured phenolic and flavonoid contents and antioxidant-related responses. Babatuyi *et al*. (2022), for example, reported that fermentation of *Citrullus vulgaris* with *Bacillus subtilis* strains produced fermented samples with higher measured phenolic and flavonoid contents and enhanced ABTS and ferric-reducing activities in selected treatments.

These findings should, however, be interpreted with caution because the analytical endpoints do not represent a single biological phenomenon. DPPH, ABTS, FRAP, and CUPRAC assays measure different chemical responses and can be affected by extraction procedures, matrix composition, and assay-specific reaction chemistry. Thus, the available evidence demonstrates changes in measured antioxidant capacity rather than establishing a universal increase in physiological antioxidant effects. Recent clinical-focused review evidence likewise emphasizes the distinction between identifying fermentation-derived bioactive compounds and demonstrating clinically relevant health outcomes (Künili *et al*., 2025). This distinction becomes particularly important when considering nutritional utilization, since reductions in antinutritional factors may improve nutrient accessibility without necessarily demonstrating increased bioavailability.

3.5 Antinutritional Factors and Nutritional Bioavailability

Reduction of antinutritional factors represents one of the important pathways through which fermentation may improve nutrient utilization. Microbial fermentation can reduce phytate, tannins, raffinose-family oligosaccharides, and other compounds that interfere with digestion or mineral utilization, while enzymatic treatment can provide additional substrate-specific hydrolysis. In the lentil beverage study, proteolytic treatment followed by *Lactobacillus* fermentation reduced phytic acid by up to 30% and substantially decreased raffinose oligosaccharides (Verni *et al*., 2020). Recent Nigerian evidence further illustrates the nutritional and functional potential of fermentation-based food formulation. Igbokwe and Igbokwe (2026) reported that fermented millet-pigeon pea-carrot flour blends showed improved protein and selected mineral and essential amino-acid characteristics with increasing pigeon-pea inclusion, while retaining functional suitability and acceptable sensory properties.

These findings depend strongly on the food matrix and microbial strain. Cereal by-products contain valuable dietary fibre, minerals, and phenolic compounds but may also contain antinutritional components and structural barriers that limit their utilization. Biological processing, including enzymatic and fermentation-based approaches, can modify these characteristics and potentially improve their food value (Luithui *et al*., 2019). Wheat-bran bioprocessing provides a further example, with enzyme and microbial treatments modifying arabinoxylans, phenolic compounds, phytic acid, and dough-related properties (Hassan *et al*., 2025).

Accordingly, future studies should move beyond measuring reductions in antinutritional compounds alone and combine such measurements with direct assessments of mineral bioaccessibility and protein digestibility. A decrease in phytate or another inhibitor does not, by itself, demonstrate improved nutrient absorption. Because these nutritional responses are closely associated with changes in the physical structure of the food matrix, the following section considers functional, physicochemical, and textural properties.

Functional, Physicochemical and Textural Properties

Enzymatic hydrolysis can modify protein solubility, water interactions, foaming, and emulsification by reducing molecular size and exposing functional groups. Studies on lupin provide direct evidence of these effects. Hydrolysis with individual enzymes improved solubility, emulsifying activity, and foaming properties, although particular protease treatments were also associated with increased bitterness (Schlegel *et al*., 2019). Enzyme combinations produced additional changes in functionality but also highlighted the trade-off between structural modification and sensory quality (Schlegel *et al*., 2020).

In cereal and fermented-beverage systems, enzymatic treatment can also modify viscosity, gel formation, and texture through changes in starch and cell-wall polymers. Xu *et al*. (2024) optimized enzymatic hydrolysis followed by fermentation in set-type oat yogurt and reported favourable acidity, cohesiveness, apparent viscosity, and exopolysaccharide production. Similarly, treatment of whole brown rice with cellulase followed by fermentation with *Lactobacillus plantarum P-8* decreased hardness and increased stickiness, indicating substantial modification of the grain structure (Lu *et al*., 2025). Quinoa protein hydrolysates have also been reported to improve yogurt quality and volatile characteristics when incorporated into a system fermented with *L. plantarum* (Chen *et al*., 2024).

These findings show that enzymes can serve not only as tools for improving nutrient release but also as means of modifying the structure and texture of food products. However, the same hydrolysis that improves solubility may weaken gel networks, while excessive degradation of cell-wall components can produce products with insufficient body or structural integrity. Process optimization should therefore be directed toward the desired properties of the final product rather than simply maximizing enzyme activity. These structural changes also have implications for flavour and sensory quality because hydrolysis and microbial metabolism can generate both desirable and undesirable compounds.

Flavour, Aroma and Sensory Properties

Flavour development is particularly sensitive to microbial strain, enzyme specificity, substrate composition, and processing sequence. In butter, enzymatic hydrolysis followed by *Lactococcus lactis* fermentation produced cheese-like flavour characteristics and a complex volatile profile, with 218 volatile compounds reported (Fan *et al*., 2025). Similarly, enzyme-assisted co-fermentation of fermented chili paste altered the volatile profile, with 166 volatile compounds detected and 19 reported to have relative odour activity values greater than one (Liu *et al*., 2026). These findings demonstrate that integrated bioprocessing can redirect flavour development through changes in precursor availability and microbial metabolism rather than simply increasing the concentration of a single nutritional component.

Sensory limitations remain an important consideration. Protein hydrolysis can generate bitter peptides, whereas fermentation may increase acidity or produce pronounced organic-acid, sulfur-containing, or ester-derived notes. Studies on lupin showed that both enzyme selection and hydrolysis intensity influenced bitterness and overall acceptability (Schlegel *et al*., 2019; Schlegel *et al*., 2020). Sensory evaluation should therefore be incorporated into process optimization rather than reserved solely for final product assessment. Because sensory responses depend strongly on processing configuration, the evidence also raises the broader question of how treatment sequence and operating conditions should be optimized for individual food matrices.

Treatment Sequence and Process Optimization

The cross-study evidence does not support a single processing sequence as optimal for all food systems. Enzymatic pretreatment before fermentation appears advantageous in several cell-wall-rich materials because it can increase the availability of fermentable substrates or release matrix-associated phytochemicals before microbial metabolism begins. This pattern is evident in studies involving citrus, microalgal, cereal, and fibre-rich materials (Li *et al*., 2024; Moirangthem *et al*., 2024; Hassan *et al*., 2025; Bilgin *et al*., 2026). However, the bee-pollen study compared alternative treatment orders and found responses that varied with the sample and enzyme preparation. It therefore should not be interpreted as evidence for a consistent advantage of enzyme-first processing (Kaškonienė *et al*., 2026).

Protein systems may behave differently because fermentation can alter protein conformation, pH, and intermediate peptide structures before protease treatment, potentially changing subsequent enzyme accessibility. In pea protein, alternative processing sequences produced different functional and antigenic responses, illustrating the importance of sequence in determining the final characteristics of the product (García-Arteaga *et al*., 2022). The practical implication is that enzyme dose, inoculum level, pH, temperature, residence time, and treatment sequence should be optimized for the specific food matrix rather than transferred unchanged from another system.

Response surface methodology, factorial designs, and related statistical approaches provide useful tools for examining interactions among these processing variables. Several reviewed studies used RSM to optimize hydrolysis or fermentation conditions, including applications involving protein hydrolysates and flavour development. Future studies should report experimental designs, factor ranges, enzyme activity units, inoculum density, kinetic endpoints, and independent validation experiments in sufficient detail to support reproducibility. Optimization is also closely connected to the choice of biological agents used in the process; therefore, the following section considers the major enzyme classes and microbial systems represented in the evidence base.

Enzyme Classes and Microbial Systems

Proteases are particularly important in protein-rich food systems because they reduce protein molecular size and facilitate the generation of peptides. The reviewed literature includes commercial preparations such as Alcalase, papain, pepsin, Flavourzyme, and other enzyme systems, together with microbial proteolysis. Studies on lupin demonstrated that Alcalase, papain, and pepsin could degrade α- and β-conglutin and improve selected functional properties, although their sensory effects differed according to the enzyme used (Schlegel *et al*., 2019; Schlegel *et al*., 2020). Recombinant microbial proteases have also been investigated for controlled plant-protein hydrolysis. For example, an aspartic protease from *Aspergillus niger* expressed in *Pichia pastoris* achieved substantial hydrolysis of soybean isolate protein (Wei *et al*., 2023).

Carbohydrases are particularly relevant to plant-derived matrices. Cellulases, pectinases, hemicellulases, xylanases, and amylases can disrupt cell-wall structures or modify starch and fibre fractions. The microbial systems represented in the evidence include *Lactiplantibacillus/Lactobacillus plantarum*, *Lactococcus lactis*, *Bacillus* species, yeasts, and filamentous fungi. Production of prolyl endopeptidase using *Trichoderma reesei* and agricultural residues further illustrates the potential for producing food-processing enzymes from renewable substrates (Liu *et al*., 2023).

At the process level, enzyme specificity and microbial metabolism should therefore be considered as interacting determinants of product formation. Recent fermentation research increasingly emphasizes selected starter cultures, controlled processing conditions, and characterization of microbial communities. Traditional fermentation can generate complex and sometimes variable metabolite profiles, whereas controlled fermentation systems may provide greater reproducibility and process control (Fitsum *et al*., 2025; Borthakur *et al*., 2026). The selection of appropriate enzymes and microorganisms also determines which underutilized substrates can be processed effectively, establishing a direct connection between bioprocessing and food by-product valorisation.

Food By-Product Valorisation and Circular Processing

The evidence base supports enzyme-assisted fermentation as a potential strategy for converting underutilized biomass into food-compatible ingredients and other value-added products. Wheat bran, brewers' spent grain, sea-buckthorn seed meal, hempseed cake, soybean residues, and other processing streams contain proteins, dietary fibre, phenolic compounds, and other potentially recoverable components that can be released or transformed through biological processing (Moirangthem *et al*., 2024; Feng *et al*., 2025; Adil *et al*., 2026). Enzymatic treatment can increase the accessibility of these components, while subsequent fermentation may stabilize, transform, or further metabolize compounds released from the matrix.

Spent coffee grounds and kitchen food waste provide broader examples of the circular-bioeconomy potential of integrated bioprocessing. Sequential pretreatment, enzymatic hydrolysis, and selective fermentation can allow different fractions of a complex waste stream to be separated and converted rather than treating the material as a single undifferentiated substrate (Cho *et al*., 2025; Arvelli *et al*.,

2026). Although some of the resulting products are intended for fuels or platform chemicals rather than direct food applications, the underlying process strategy is relevant to food manufacturing because it demonstrates the potential value of sequential fractionation and targeted bioconversion.
Claims regarding sustainability should nevertheless be evaluated at the level of the complete process rather than attributed automatically to the use of enzymes or microorganisms. Enzyme production, purification, formulation, transportation, water consumption, energy requirements, downstream separation, and residual waste can all contribute to the overall environmental burden. Biological processing is therefore not inherently sustainable simply because it uses biological agents. This whole-process perspective also highlights the importance of safety, standardization, and regulatory compliance when laboratory-scale processes are considered for food applications.

Safety, Standardization and Regulatory Considerations

Fermentation can contribute to food safety through acidification, competition with undesirable microorganisms, and production of inhibitory metabolites, but fermentation alone does not guarantee product safety. Potential risks include contamination by undesirable microorganisms, formation of biogenic amines, mycotoxin-related hazards, and uncontrolled accumulation of other metabolites. The safety profile therefore depends on the microorganism used, substrate composition, process conditions, hygienic controls, and subsequent storage and handling (Fitsum *et al*., 2025).
Enzymatic processing also requires appropriate control of enzyme identity, purity, activity, dosage, reaction conditions, and residual enzyme levels where relevant. Commercial enzyme preparations may contain additional components, and their suitability for food applications depends on the intended use and applicable regulatory requirements. Similarly, the use of microbial cultures requires appropriate strain identification, characterization, and process control to ensure that beneficial fermentation does not introduce unintended hazards (Abdullahi *et al*., 2021).
Standardization remains a major challenge across the evidence base. Studies frequently differ in enzyme dosage units, substrate-to-enzyme ratios, inoculum levels, fermentation duration, pH, temperature, extraction procedures, and analytical methods. Such differences make direct comparison of outcomes difficult and may partly explain the variation reported across food matrices. Future studies would benefit from reporting standardized process parameters and using validated analytical methods alongside appropriate untreated, enzyme-only, and fermentation-only controls (García-Arteaga *et al*., 2022; Xu *et al*., 2024; Moirangthem *et al*., 2024).
For translation into commercial food systems, safety assessment must also be considered together with sensory acceptability, process reproducibility, economic feasibility, regulatory requirements, and scale-up performance. A treatment that produces a favourable biochemical response at laboratory scale may not remain practical or economically viable when applied to larger volumes. Consequently, the future development of enzyme-assisted fermentation should focus not only on maximizing biochemical transformation but also on establishing controlled, reproducible, safe, and economically feasible processes suitable for real food production. Controlled starter cultures, hygienic handling, monitoring of pH and temperature and finished-product testing are therefore necessary (Fitsum *et al*., 2025).
Enzyme safety also requires consideration of the source organism, production method, purity, residual activity, and regulatory status of the enzyme preparation. In protein hydrolysates, hydrolysis may reduce the immunoreactivity of some proteins, but it should not be assumed that allergenicity is completely eliminated. Studies on lupin have shown that selected hydrolysis strategies can modify major IgE-reactive proteins, although both allergenicity-related responses and sensory characteristics depend on the enzyme used and the intensity of processing (Schlegel *et al*., 2019; Schlegel *et al*., 2020).
Standardization remains a major limitation across the evidence base. Some studies report enzyme concentrations without specifying activity units, while others describe fermentation conditions without providing inoculum density or viable-cell counts. Differences in extraction procedures, reporting bases, and antioxidant-assay expression also make comparisons between studies difficult. Future publications should therefore provide sufficient process information, appropriate controls, and validated analytical methods. These reporting limitations have implications for scale-up because laboratory performance cannot be reliably reproduced at larger scale when process parameters and analytical endpoints are incompletely defined (García-Arteaga *et al*., 2022; Xu *et al*., 2024; Moirangthem *et al*., 2024).

Scale-up, Enzyme Engineering and Emerging Technologies

Despite the progress reported in laboratory studies, performance at small scale does not necessarily predict behaviour during industrial processing. Food matrices are heterogeneous, viscosity may change during hydrolysis and fermentation, and mass-transfer limitations can affect enzyme accessibility and microbial activity. Enzyme stability, production cost, purification, storage, and recovery or reuse also remain important considerations. Enzyme immobilization may improve operational stability and facilitate enzyme recovery, while recombinant production offers opportunities to obtain enzymes with selected catalytic characteristics (Mora & Toldrá, 2023; Santos-Sánchez *et al*., 2024).
The wider literature also points toward precision fermentation, computational optimization, and multi-omics approaches as emerging tools for improving process control. Artificial intelligence and machine-learning methods may assist with enzyme screening, prediction of enzyme-substrate interactions, fermentation optimization, and the design of multi-enzyme systems. Similarly, omics-based approaches can help link microbial genes and enzyme functions with metabolite production and changes in food composition. These technologies should, however, complement rather than replace experimental validation (Farias & Kempka, 2026).
Precision fermentation provides a related emerging route for controlled microbial production of targeted food ingredients and products. Recent work has highlighted its potential applications in food production, sustainability and process innovation, while also emphasizing technological, regulatory and economic challenges (Salihu *et al*., 2026). Adaptive process control represents another promising direction. Real-time monitoring of parameters such as pH, temperature, enzyme activity, and microbial growth could reduce batch-to-batch variation and improve product consistency. Nevertheless, practical adoption will depend on equipment requirements, operating costs, regulatory considerations, technical expertise, and consumer acceptance. Technological innovation should therefore be evaluated alongside safety, reproducibility, application-specific performance, and economic feasibility rather than on technological novelty alone.

Contextual and Complementary Evidence from the Wider Evidence Base

The wider evidence base provides additional context for understanding protein hydrolysis, peptide generation, and functional-food development. Reviews addressing soybean functional peptides, plant-protein bioactive peptides, dairy peptides, oat peptides, and fermentation-derived peptides consistently emphasize the importance of enzyme specificity, microbial proteolysis, peptide size and sequence, and subsequent bioaccessibility in determining the functional characteristics of hydrolysates (Tadesse & Emire, 2020; Akbarian *et al*., 2022; Majura *et al*., 2022; Murtaza *et al*., 2022; Rafique *et al*., 2022; Zhu *et al*., 2023; Zhao *et al*., 2024; Zhao *et al*., 2025; Farias & Kempka, 2026).

Additional literatures illustrate the range of applications associated with enzyme-assisted processing beyond the principal food-quality studies. Enzymatic pretreatment followed by fermentation has been investigated using sugarcane bagasse, vinegar-production systems, and rapeseed cake, with applications ranging from xylitol and organic-acid production to improved conversion of protein-rich residues (Gai *et al*., 2022; Song *et al*., 2022; Singkhum & Tanganurat, 2026). Separately, enzyme-assisted extraction of soybean-hull polysaccharides provides complementary evidence on the recovery and functional properties of carbohydrate fractions but does not constitute a combined fermentation study (Song *et al*., 2022). Other protein-processing studies have examined whey bioactive peptides, soybean-protein modification, and hydrolysate production, while enzyme-focused reviews describe technological approaches for producing value-added whey ingredients (Zhang *et al*., 2023; Irazoqui *et al*., 2024; Sun, M. *et al*., 2024; Idowu & Yupanqui, 2025; Luparelli *et al*., 2025; González-Quijano *et al*., 2026).

The evidence base also contains literature relevant to food safety, valorisation, and process boundaries. Recent research on egg-white biotechnology highlights the importance of integrating processing with microbiological and safety controls, whereas agro-industrial fibre-rich by-products and plant residues have been investigated as substrates for microbial conversion into functional beverages and other value-added materials (Sentís-Moré *et al*., 2025; Mannott *et al*., 2026). Studies involving peanut meal, Astragalus stems, and faba-bean polyphenols further demonstrate that combined or fermentation-based processing can modify antinutritional factors, phenolic profiles, and antioxidant responses. However, some of these applications are directed toward animal feed or ingredient valorisation rather than direct human-food production and should therefore not be generalized without considering their intended application (Liu *et al*., 2024; Chen *et al*., 2025; Ning *et al*., 2026).

The identified records also include animal-feed and matrix-specific fermentation studies. Cellulase combined with lactic-acid bacteria has been evaluated in mixed silages, providing contextual evidence on how enzyme-microbe interactions influence fermentation quality and microbial communities (Ju *et al*., 2023; Li *et al*., 2024; Li *et al*., 2025). Fermented goat milk and whey-protein systems provide additional evidence relating to peptide generation and measured ACE-inhibitory, antioxidant, or anti-inflammatory activities (Rubak *et al*., 2022; Moon & Kim, 2026). Earlier studies on soy-protein hydrolysis remain relevant for interpreting the relationship between hydrolysis conditions and functional-property changes, while more recent whey-hydrolysate research extends these concepts to value-added processing (Sun, 2011; Irazoqui *et al*., 2024).

Taken together, these contextual studies help define the boundaries of the core evidence and clarify which findings can reasonably be generalized across food matrices and which remain specific to particular substrates or applications. The combined evidence therefore supports a consistent interpretation: enzyme-assisted fermentation has broad technological potential, but its effects depend on the substrate, enzyme system, microorganism, treatment sequence, and operating conditions. The central challenge is consequently not whether biological processing can modify food matrices, but whether desirable changes can be reproduced consistently and translated into safe, acceptable, economically viable, and scalable food applications.

Research Gaps and Future Directions

Several important research gaps emerge from the reviewed evidence. Direct head-to-head comparisons of enzyme-only, fermentation-only, combined enzyme-fermentation, fermentation-enzyme, and simultaneous enzyme-microbe systems remain limited. Without comparable controls, it is difficult to determine whether an observed improvement results from the integrated treatment, one component alone, or an interaction between the two processes.

Future experimental studies should therefore report enzyme activity units, enzyme-to-substrate ratios, inoculum density, viable-cell counts, pH, temperature, processing time, and treatment sequence in sufficient detail to permit meaningful comparison and reproduction.

Protein-focused studies should combine degree of hydrolysis with molecular-weight distribution, peptide sequencing, simulated gastrointestinal digestion, and bioaccessibility measurements. This would provide a stronger basis for linking structural changes in proteins with potential nutritional or functional effects.

Studies of antioxidant activity should report assay conditions, concentration basis, extraction procedures, and sample preparation clearly. Different antioxidant assays should be interpreted as complementary measurements rather than interchangeable indicators of physiological antioxidant activity.

Research on antinutritional factors should likewise combine concentration measurements with direct assessments of mineral bioaccessibility and protein digestibility. A reduction in an antinutritional compound alone is insufficient to demonstrate improved nutritional utilization.

Sensory evaluation should be integrated into process optimization, particularly in systems where proteolysis or fermentation may generate bitterness, excessive acidity, undesirable aromas, or textural changes. Optimization should therefore consider nutritional, technological, and sensory responses simultaneously.

Scale-up investigations should quantify enzyme cost, stability, recovery or reuse, fermentation kinetics, contamination control, energy requirements, downstream processing, and batch-to-batch reproducibility. Such information is essential for determining whether laboratory-scale improvements are economically and technically transferable to commercial production.

Where health-related claims are proposed, appropriately designed animal and human studies will be required before findings based solely on in vitro ACE-inhibitory, antioxidant, or antidiabetic assays are interpreted as evidence of physiological efficacy.

Research in Nigeria and other food-insecure settings should place greater emphasis on locally available cereals, legumes, fruits, and agro-industrial residues. Such studies should consider affordability, equipment requirements, process

simplicity, cultural acceptability, locally adapted starter cultures, and the availability of appropriate analytical facilities.

Future evidence syntheses would also benefit from prospectively registered protocols, comprehensive database searches, complete search strings, transparent screening records, and structured risk-of-bias assessment. These measures would strengthen reproducibility and improve confidence in the conclusions drawn from the expanding literature.

Strengths and Limitations of the Review

A major strength of this review is the use of a defined initial record set comprising 82 records, allowing recent primary studies to be considered alongside mechanistic and contextual literature. The final manuscript uses 73 publications in total: 69 publications retained through the screening process plus four supplementary contextual publications identified during the iterative synthesis. The synthesis also distinguishes direct evidence from combined enzymatic-microbial processing from single-process studies and non-food applications. This distinction reduces the risk of attributing an observed effect to combined treatment when the two interventions were not experimentally evaluated together.

The 69 screened-in publications should not be interpreted as an exhaustive representation of the global literature, and the four supplementary contextual publications were added during the iterative synthesis rather than through the original screening flow. The evidence base was also heterogeneous with respect to substrates, enzymes, microorganisms, processing conditions, experimental designs, and analytical methods, which prevented a defensible quantitative meta-analysis.

In addition, several studies assessed in vitro activities or laboratory-scale processes. Such findings require caution when extrapolating to human health outcomes, commercial feasibility, or environmental sustainability. These limitations support the use of a narrative, thematic synthesis rather than quantitative pooling and should be considered when interpreting the conclusions of the review.

CONCLUSION

The literatures reviewed indicates that integrated enzymatic hydrolysis and microbial fermentation represent flexible bioprocessing strategies capable of modifying food matrices at multiple structural and biochemical levels. Combined processing can improve selected aspects of protein digestibility and functionality, release or transform phenolic and flavonoid compounds, reduce selected antinutritional factors, modify texture, and contribute to the development of new aroma and flavour profiles.

However, the magnitude and direction of these effects are strongly matrix-dependent rather than universal. Enzymatic pretreatment followed by fermentation was effective in several cell-wall-rich matrices, whereas fermentation followed by enzymatic hydrolysis produced pronounced changes in selected protein systems. Findings from bee pollen further demonstrate that the preferred sequence may depend on the enzyme preparation and characteristics of the substrate. Accordingly, there is no single processing configuration that can be considered optimal across all food systems.

Successful application therefore depends on matching the substrate, enzyme system, microorganism, treatment sequence, and operating conditions to the desired characteristics of the final product. Translation of these approaches into functional foods and sustainable food-processing systems will require standardized reporting, direct comparisons of alternative processing sequences, validated assessments of digestibility and bioaccessibility, integrated sensory evaluation, appropriate safety controls, scale-up studies, and economic assessment. Where health benefits are proposed, physiological validation through appropriately designed animal and human studies will also be necessary. Overall, the evidence supports enzyme-assisted fermentation as a promising but context-dependent bioprocessing strategy whose future development will depend on improving reproducibility, process control, safety, scalability, and application-specific performance.

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