Restructuring for Excellence: A New Era for the Pakistan Journal of Food Sciences

Nauman Khalid[1,2]

[1]*Department of Food Science and Technology, School of Food and Agricultural Sciences, University of Management and Technology, Lahore 54000, Pakistan*

[2]*College of Health Sciences, Abu Dhabi University, Abu Dhabi, 59911 United Arab Emirates*

Correspondence: Nauman Khalid (nauman.khalid@umt.edu.pk; nauman.khalid@adu.ac.ae)

ORCIDs: Nauman Khalid (https://orcid.org/0000-0002-8045-199X)

Food security, climate-induced agricultural disruptions, and a rising triple burden of malnutrition present critical challenges to Pakistan. Addressing these crises requires robust, evidence-based scientific solutions. As the premier national platform for food science literature, the Pakistan Journal of Food Sciences (PJFS) must evolve to meet these contemporary demands. This editorial marks the official relaunch of PJFS, introducing a modernized editorial framework, a reconstituted board of experts, and a strategic roadmap designed to elevate Pakistani food and nutrition research to global prominence.

Pervasive Challenges in Pakistani Food Research

To effectively uplift the national landscape, food research in Pakistan must directly confront several structural and systemic bottlenecks. A primary challenge is the critical disconnect between academic research and industrial application, which frequently leaves valuable laboratory findings trapped in uncommercialized theses. Furthermore, the localized food sector suffers from a severe deficit in validated baseline data regarding chemical residues, microplastic contamination, and heavy metal bioaccumulation across the food supply chain. This lack of data is worsened by limited access to high-end analytical equipment, such as LC-MS/MS and NMR spectroscopy, which restricts researchers to basic proximate analyses rather than deep molecular or mechanistic investigations. Finally, the absence of standardized, uniform protocol replication across different provincial laboratories undermines data reproducibility, making it difficult for Pakistani food research to meet the stringent validation standards required by top-tier international journals.

Strengthening Editorial Governance

A rigorous peer-review mechanism is the foundation of scientific integrity. To accelerate processing times without compromising quality, PJFS has transitioned to a fully digital, double-blind peer-review workflow. The journal has reconstituted its Editorial Board to balance deep national expertise with international perspectives. The new board comprises leading scientists

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specializing in post-harvest technology, food safety microbiology, functional foods, and public health nutrition. These experts are tasked with enforcing strict methodological compliance, ensuring data reproducibility, and eliminating predatory publishing practices. Furthermore, PJFS is implementing a strict ethical policy aligned with the Committee on Publication Ethics (COPE) guidelines. Every submission will undergo rigorous plagiarism screening and mandatory disclosure of conflicts of interest to safeguard the credibility of our published science.

Strategic Priorities for Research Upliftment

To enhance the global impact and citation footprint of Pakistani research, PJFS will prioritize high-utility, cross-disciplinary studies. Our editorial strategy focuses on four critical pillars: climate-resilient post-harvest systems, nutritional biomarkers and biofortification, indigenous food value-added processing, and food safety regimes. We will heavily emphasize innovations that minimize post-harvest losses in Pakistan's specific supply chains and champion agronomic and genetic solutions addressing local micronutrient deficiencies such as stunting, wasting, and anemia. Additionally, the journal will focus on modernizing traditional Pakistani foods through value-addition and functional property validation, while supporting local regulatory authorities in mitigating chemical and microbial contaminants. The journal will also actively solicit review articles that synthesize regional data, offering clear policy insights for government and industrial stakeholders.

The Stewardship of PSFST and Global Alignment

The revitalization of PJFS is intimately tied to its parent organization, the Pakistan Society of Food Scientists and Technologists (PSFST), which serves as the foundational bedrock for national advocacy, professional networking, and capacity building. PSFST bridges the gap between academia, regulatory bodies, and corporate food entities, providing the essential industry-wide momentum needed to sustain this journal relaunch. Moving forward, a core strategic objective of PSFST and PJFS is to forge structured alignments and formal partnerships with premier international bodies, including the Institute of Food Technologists (IFT) in the USA, the International Union of Food Science and Technology (IUFoST), and the European Federation of Food Science and Technology (EFFoST). Through joint international symposia, cross-border research exchanges, and shared technical committees, these global alliances will expose Pakistani researchers to cutting-edge international standards, co-authored global grants, and globally synchronized food safety protocols.

Introducing the New Editorial Leadership Team

The strategic relaunch and structural overhaul of the journal are driven by a reconstituted team of distinguished visionaries and academics who bring profound expertise from Pakistan's leading food and nutritional science institutions. Serving as Patron-in-Chief is Prof. Dr. Faqir Muhammad

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Anjum (SI, FPAS), the former Vice Chancellor of the University of The Gambia. Dr. Anjum provides overarching institutional stewardship, utilizing his decorated background in academic governance to ensure that the journal adheres closely to national scientific mandates while pushing for global research boundaries. His pioneering research focuses on advanced grain science, cereal technology, and national nutritional policy, with a specific emphasis on optimizing the milling and baking quality of indigenous wheat varieties to combat regional micronutrient deficiencies.

Leading the intellectual direction of the journal is Editor-in-Chief Prof. Dr. Tahir Zahoor, from the Faculty of Applied Sciences at NUR International University, Lahore. As the primary academic authority, Dr. Zahoor's vision centers on enforcing publication ethics, elevating research standards, and aligning submissions with contemporary challenges in national food security. His specialized research focus covers food microbiology, dairy technology, and functional food development, with significant impact achieved through isolating indigenous probiotic strains for therapeutic foods and establishing strict microbial safety barriers in local dairy supply chains.

Directing day-to-day operations is Managing Editor Prof. Dr. Nauman Khalid, from the School of Food and Agricultural Sciences at the University of Management and Technology, Lahore. Dr. Khalid oversees the transition into a fully digital, double-blind peer-review ecosystem, ensuring high efficiency in technical workflows, production cycles, and author communications. His advanced research targets the nano-encapsulation of bioactive compounds, food safety, and novel extraction techniques, specializing in developing lipid-based delivery systems to fortify everyday foods with unstable essential vitamins and antioxidants.

Supporting the technical evaluation is a panel of distinguished Associate Editors who manage specific domain-level tracks, drive the intellectual evaluation of assigned manuscripts, and facilitate strict quality controls for breakthrough work in food science applications. Among them is Associate Editor Prof. Dr. Muhammad Issa Khan, representing the National Institute of Food Science and Technology at the University of Agriculture, Faisalabad. Dr. Khan's research focuses on meat science, antioxidant active packaging, and functional product formulation, aiming to extend the shelf-life of perishable proteins using natural plant extracts to directly reduce post-harvest supply chain losses. Working alongside him to manage specific domain tracks is Associate Editor Prof. Prof. Dr. Amna Sehar from the Department of Food Engineering, University of Agriculture, Faisalabad. Her work focuses on advanced food preservation, nutrient fortification, and spectroscopic techniques for food safety. The Associate Editor, Prof. Dr. Mian Kamran Sharif, research focus on fortification of foods, especially wheat, rice, processed foods, composite flour technology, and food allergens. The other prominent Associate Editors include: Dr. Muhammad Jahangir, Dr. Hafiz Ubaid-ur-Rahman, and Dr. Ahmed Mujtaba.

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Visibility and Global Integration

The ultimate benchmark of PJFS's success will be its integration into top-tier global indexing databases. Our immediate three-year roadmap focuses on securing indexing in Scopus and Web of Science (Science Citation Index Expanded). To achieve this, the journal is moving to a continuous publication model, ensuring that accepted papers are immediately discoverable online. We are launching an Open Access framework to remove paywalls, making Pakistani research highly accessible to international scholars, policymakers, and industry leaders. By upgrading our technical infrastructure, enforcing world-class editorial standards, and focusing on high-impact local solutions, PJFS will serve as the definitive catalyst for advancing food and nutrition science in Pakistan and beyond.

Official Call for Papers

In alignment with our structural reorganization, the PJFS officially invites researchers, academicians, and food industry professionals to submit their original research articles, short communications, and comprehensive review papers for our upcoming relaunch volume. We welcome high-impact submissions addressing urgent national and global concerns, particularly in climate-smart agriculture value chains, public health nutrition, novel food processing, and regulatory food safety frameworks. All submissions must represent original work that has not been published or considered elsewhere, and they must comply strictly with the updated PJFS structural formatting guidelines. Authors can benefit from a streamlined digital submission portal, transparent double-blind peer review, and accelerated post-acceptance online publication under our new continuous publication model. Complete submission guidelines, ethical policy compliance checklists, and access to the online editorial management portal are available through the official PJFS journal website.

To sustain our newly adopted Open Access framework and eliminate systemic barriers to global knowledge exchange, PJFS has instituted a sustainable yet equitable financial architecture. Operating as a non-profit academic vehicle under the aegis of PSFST, the journal levies a subsidized Article Processing Charge (APC) of PKR 25,000 for local domestic institutional submissions, and USD 150 for regular international submissions, which directly covers technical typesetting, digital object identifier (DOI) registration, and persistent server hosting fees. However, recognizing financial constraints faced by resource-strained researchers, the journal enforces a strict, transparent waiver matrix aligned with global scientific equity standards. Submissions from primary corresponding authors based in low-income economies, as classified by the United Nations and World Bank economic definitions, will automatically receive a full 100% publication fee waiver. Furthermore, to incentivize high-quality local science, full or partial waivers will be granted dynamically to postgraduate students, independent researchers without institutional grant funding, or for meritorious manuscripts selected directly by the Editor-in-Chief based on extraordinary research impact.

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Effect of *Moringa oleifera* Leaf Powder Level on the Proximate Composition and Sensory Acceptance of Roasted Chickpea Bars

Ali Hussnain Sajid[1]*, Amir Arshad[1], and Saliha Ashfaq[1]

[1]Department of Food Science and Technology, Riphah International University, Faisalabad, Pakistan

***Corresponding author:** 1947hussnain@gmail.com

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Abstract

This screening study evaluated roasted chickpea bars in which *Moringa oleifera* leaf powder (MLP) replaced 0%, 2%, 4%, or 6% of a 250-g chickpea-MLP base while jaggery and cocoa spread remained fixed. These levels corresponded to 0%, 1.35%, 2.70%, and 4.05% MLP in the complete 370-g formulation. Four treatments (T_o-T_3) were assessed for crude fat, crude protein, moisture, ash, and six sensory attributes on a nine-point scale. In the supplied results, protein increased from 25.6% in T_o to 25.9% in T_3, ash increased from 3.24% to 3.44%, and moisture decreased from 8.26% to 7.51%. Fat varied non-monotonically from 27.9% to 28.3%. Treatment differences were reported for each proximate variable ($p < 0.05$). Among the sensory attributes, only the moringa-flavour item was reported to differ significantly; overall acceptability did not differ significantly. T_1 had the highest overall acceptability score (7.66 ± 0.57), whereas T_3 had the highest protein and ash values. Thus, T_1 may be advanced as a candidate for confirmatory testing, but the data do not establish a statistically superior or optimized formulation. Independent batch replication, validated analytical methods, panel characteristics, ethics documentation, and the statistical model must be confirmed before inferential conclusions are accepted.

Keywords: Chickpea-based bar; *Moringa oleifera;* Leaf powder; Proximate composition; Sensory acceptance; Formulation screening

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1. Introduction

Chickpea *(Cicer arietinum L.)* is a pulse valued for protein, dietary fibre, starch, vitamins, and minerals. Its nutritional composition and wide availability make it a useful base for plant-forward convenience foods (Jukanti et al., 2012). Roasting also changes chickpea colour, flavour, and functional properties and can therefore influence its performance in ready-to-eat products (Jogihalli et al., 2017).

Moringa oleifera leaves contain protein and mineral matter as well as multiple phytochemicals, but their composition depends on cultivar, maturity, processing, and storage. MLP has consequently been investigated as a natural food fortificant. Fortification can improve selected compositional attributes while also changing colour, flavour, and texture; formulation therefore requires an explicit balance between composition and acceptability (Stohs & Hartman, 2015; Vishwakarma et al., 2022).

Snack bars provide a convenient matrix for combining cereal, pulse, fruit, and binding ingredients. Previous work has shown that formulation strongly affects the physicochemical and sensory properties of cereal and protein bars, including bars containing roasted chickpea flour (Jabeen et al., 2022; Samakradhamrongthai et al., 2021). However, evidence remains limited for roasted chickpea bars in which low levels of MLP directly replace part of the chickpea component while jaggery and cocoa spread remain fixed.

The objective was therefore to formulate roasted chickpea bars containing 0-6% MLP in the chickpea-MLP base and compare their reported proximate composition and sensory scores. The primary outcomes were overall acceptability and crude protein; moisture, ash, crude fat, and the remaining sensory attributes were secondary outcomes. It was hypothesized that increasing MLP would increase protein and ash and would alter sensory perception. Because only four inclusion levels were examined, the study was treated as exploratory formulation screening rather than definitive optimization.

2. Materials and Methods

2.1 Raw materials and moringa-leaf preparation

Roasted chickpeas, jaggery, and cocoa spread were purchased from retail markets in Chiniot, Pakistan, and transported to the Food Processing Laboratory, Riphah International University, Faisalabad Campus. Mature *M. oleifera* leaves were collected from a healthy plant, washed, shade-dried, milled, and used as MLP.

2.2 Formulation and experimental design

A single-factor formulation design was used, with MLP inclusion as the factor and four levels (0%, 2%, 4%, and 6%). The supplied treatment plan indicates that MLP replaced chickpea within a 250-

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g chickpea-MLP base, whereas jaggery and cocoa spread were held constant at 60 g each. The reconstructed mass formulation is shown in Table 1.

Table 1. Reconstructed formulation of the chickpea-moringa bars

Treatment	MLP in 250-g base (%)	Roasted chickpea (g)	MLP (g)	Jaggery (g)	Cocoa spread (g)	MLP in complete formulation (%)
T_o	0	250	0	60	60	0.00
T_1	2	245	5	60	60	1.35
T_2	4	240	10	60	60	2.70
T_3	6	235	15	60	60	4.05

MLP, *Moringa oleifera* leaf powder. Percentages are expressed on the 250-g chickpea-MLP base unless otherwise specified. The reconstructed starting mass was 370 g for every treatment.

2.3 Bar preparation

Roasted chickpeas and MLP were weighed according to the treatment plan. Chickpeas were coarsely ground. Jaggery was heated to 80-90 °C and blended with cocoa spread to form a binder. The chickpea and MLP mixture was added gradually and stirred while the mass was heated over low heat for 3-5 min until semi-solid. The mixture was spread in a greased, parchment-lined tray, cooled at room temperature for 30-40 min, cut into bars, wrapped in aluminium foil, and retained for analysis. This general preparation sequence is consistent with published cereal-bar processing approaches (Samakradhamrongthai et al., 2021).

2.4 Proximate analyses

Finely ground bar samples were analysed for moisture, ash, crude protein, and crude fat. Results were expressed as percentages by mass on the as-is basis. The original draft did not identify validated method numbers; these must be supplied so that the work can be reproduced and checked against the current AOAC compendium (AOAC International, 2023).

2.4.1 Moisture

A 2-3 g test portion was dried in a hot-air oven at 105 °C to constant mass. Moisture (%) was calculated as 100 × (initial test-portion mass − dried mass)/initial test-portion mass.

2.4.2 Ash

A test portion was incinerated in a muffle furnace at 550 °C for 4-6 h, or until complete ashing, cooled in a desiccator, and weighed. Ash (%) was calculated as 100 × ash residue mass/test-portion mass.

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2.4.3 Crude protein

Total nitrogen was determined by Kjeldahl digestion, distillation, and titration. Crude protein was calculated as nitrogen (%) multiplied by the conversion factor used for the analysis.

2.4.4 Crude fat

Crude fat was described in the original records as a gravimetric extraction. Fat (%) was calculated as 100 × mass of recovered extract/test-portion mass. The earlier label 'Gerber method' has been removed because it conflicted with the stated gravimetric procedure and is not, without further adaptation, a sufficient description for this solid food matrix.

2.5 Sensory evaluation

Appearance, flavour, texture, chewiness, moringa-flavour liking, and overall acceptability were evaluated on a nine-point hedonic scale anchored from 1 (dislike extremely) to 9 (like extremely). A controlled hedonic test should document participant recruitment, serving conditions, coding, order randomization, palate cleansing, and consent in accordance with accepted sensory practice (ISO 11136:2014/Amd 1:2020).

2.6 Statistical analysis

Each response was summarized as mean ± standard deviation. The treatment effect was assessed by one-way analysis of variance, with pairwise mean separation at $p < 0.05$. For valid inference, independently prepared batches not repeated readings from the same batch must be the experimental units for composition. Sensory observations must likewise be analysed with participant dependence accounted for when each participant rates all formulations.

3. Results and Discussion

3.1 Proximate composition

The reported proximate results are presented in Table 2. All four variables were reported to differ among formulations at $p < 0.05$; however, the inferential results remain provisional until batch replication and the post-hoc procedure are confirmed.

Table 2. Reported proximate composition of chickpea bars containing 0-6% MLP

Treatment	Crude fat (%)	Crude protein (%)	Moisture (%)	Ash (%)
T_o (0%)	27.9 ± 0.01^d	25.6 ± 0.01^d	8.26 ± 0.01^d	3.24 ± 0.05^d
T_1 (2%)	28.3 ± 0.05^a	25.7 ± 0.01^c	7.91 ± 0.02^c	3.31 ± 0.00^c
T_2 (4%)	28.1 ± 0.05^c	25.8 ± 0.01^b	7.77 ± 0.01^b	3.37 ± 0.01^b
T_3 (6%)	28.2 ± 0.05^b	25.9 ± 0.02^a	7.51 ± 0.02^a	3.44 ± 0.05^a

Values are the means ± SD supplied in the original manuscript. Insert n. Within a column, unlike superscripts were reported to indicate $p < 0.05$; confirm the post-hoc test and experimental unit.

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Protein increased monotonically by 0.3 percentage point from T_o to T_3, and ash increased by 0.20 percentage point. These directions are compatible with replacing part of the chickpea base with a leaf powder containing protein and mineral matter, but the effect sizes are small and do not identify individual amino acids or minerals. Accordingly, ash should not be described as a direct measure of nutritional mineral quality. Studies of natural food fortification and chickpea-containing bars similarly show that compositional effects depend on both the fortificant and the full product matrix (Jabeen et al., 2022; Vishwakarma et al., 2022).

Moisture declined by 0.75 percentage point between T_o and T_3. This result may reflect changes in formulation, water binding, heating, or sample handling, but no water activity, microbial counts, packaging comparison, or storage study was performed. The observed moisture difference therefore cannot support a shelf-life claim.

Crude fat showed no dose-response pattern: T_1 had the highest mean, T_2 was lower, and T_3 was intermediate. The small separation among means, together with the unresolved extraction method and replication, makes a causal explanation based on MLP lipid content premature.

3.2 Sensory scores

Table 3 shows that all formulations had mean overall-acceptability scores above 6 on the nine-point scale. Only the moringa-flavour item was reported to differ significantly. No statistically significant difference was reported for appearance, flavour, texture, chewiness, or overall acceptability.

Table 3. Reported sensory scores of chickpea bars containing 0-6% MLP

Treatment	Appearance	Flavour	Texture	Chewiness	Moringa-flavour liking	Overall acceptability
T_o (0%)	7.00 ± 1.00	7.00 ± 1.73	6.66 ± 0.57	8.00 ± 1.00	5.00 ± 0.00^b	7.33 ± 1.15
T_1 (2%)	7.30 ± 1.15	6.66 ± 0.57	7.00 ± 1.73	8.00 ± 1.00	8.33 ± 0.60^a	7.66 ± 0.57
T_2 (4%)	8.00 ± 1.00	8.00 ± 1.00	8.33 ± 0.57	6.30 ± 1.15	7.00 ± 1.00^a	6.66 ± 0.57
T_3 (6%)	7.30 ± 1.15	8.33 ± 0.57	8.33 ± 1.15	7.30 ± 1.02	6.67 ± 0.60^{ab}	7.33 ± 1.15

Values are the means ± SD supplied in the original manuscript. Insert n. Unlike superscripts in the moringa-flavour column were reported to indicate $p < 0.05$; the other attributes were reported as non-significant. The label 'moringa-flavour liking' assumes that this item used the same hedonic anchors; confirm before submission.

T_2 had the highest numerical appearance score, T_3 the highest flavour score, T_2 and T_3 the highest texture scores, and T_o and T_1 the highest chewiness scores. T_1 had the highest numerical overall-

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acceptability score, but its mean was not statistically distinguishable from the other treatments. The data therefore support acceptable average ratings across the tested range, not superiority of one formulation.

The moringa-flavour item was highest for T_1 and lower for T_3. If this was a hedonic item, the pattern suggests that moderate MLP inclusion was liked more than the control or the highest inclusion level. If it was an intensity item, however, the analysis and interpretation must be rewritten because intensity is not equivalent to liking. A larger, well-characterized consumer test with randomized serving order is needed to estimate preference and to test whether 2% MLP is robustly preferred (ISO 11136:2014/Amd 1:2020).

3.3 Candidate formulation and study limitations

On a descriptive basis, T_1 combines the highest mean overall acceptability with slightly higher protein and ash than T_o. It may therefore be advanced as a candidate for confirmatory testing. It should not be described as a statistically optimized formulation because overall acceptability did not differ significantly and the experiment did not use an optimization model or a sufficiently resolved concentration range.

Interpretation is limited by the missing documentation of independent batches, the unresolved analytical methods, and the absent panel description and ethics statement. The study also did not measure dietary fibre, individual minerals, vitamins, phenolics, antioxidant activity, instrumental colour or texture, water activity, microbial quality, nutrient bioavailability, storage stability, or serving-level nutrition. Consequently, the data establish only the reported proximate and sensory outcomes under the tested conditions; they do not establish therapeutic, disease-prevention, or shelf-life benefits.

4. Conclusion

Replacing 0-6% of the chickpea-MLP base with MLP was associated with small increases in the reported protein and ash values and a decrease in moisture, while crude fat changed non-monotonically. Mean sensory scores remained above 6 across treatments, and only the moringa-flavour item was reported to differ significantly. T_1 (2% of the 250-g base; 1.35% of the complete formulation) had the highest numerical overall-acceptability score and is a reasonable candidate for further development, but the present data do not demonstrate a statistically superior optimum. Before publication, the authors should verify the formulation basis and raw data, report independent replication and validate analytical methods, and supply complete sensory and statistical procedures. Confirmatory work should include an adequately powered consumer test, instrumental quality measures, nutrient-specific analyses, and a designed storage study.

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Eating Disorders in Youth: A Systematic Review of Clinical Profile, Prevalence, and Psychosocial Impacts

Hafsa Naseer[1], Muhammad Tauseef Sultan[1], Ahmad Mujtaba Noman[1*], Zainab Ahmed[1], Shehnshah Zafar[1], Sabeen Zahra[1], Muhammad Abdul Rahim[2], Hassan Raza[1*]

[1] Faculty of Food Science and Nutrition, Bahauddin Zakariya University, Multan 60800, Pakistan

[2] Department of Food Science & Nutrition, Faculty of Medicine and Allied Health Sciences, Times University, Multan 60700, Pakistan

Corresponding Authors: ahmadmujtaba493@gmail.com; sh.raza40@yahoo.com

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Abstract

Eating disorders (EDs), including anorexia nervosa (AN), bulimia nervosa (BN), and binge-eating disorder (BED), are serious psychiatric conditions increasingly affecting adolescents and young adults. This systematic review synthesized evidence on prevalence, age at onset, developmental trajectories, comorbidities, and healthcare disparities following PRISMA 2020 and MOOSE guidelines. Eighteen quantitative and mixed-methods studies published during 2016–2025 were identified through PubMed, Google Scholar, and PsycINFO using predefined eligibility criteria. Lifetime prevalence in Western countries was 0.16% for AN, 0.63% for BN, and 1.53% for BED. Prevalence was substantially higher among females (8.6–17.9%) than males (2.2–4.1%). Typical onset occurred at 10–20 years for AN, 13–20 years for BN, and 12–24 years for BED. BMI trajectories diverged early: AN was associated with lower BMI by age 4 in girls and age 2 in boys, whereas BN and BED were linked to higher BMI by age 6. Trauma, particularly sexual and emotional abuse, increased the likelihood of binge–purge presentations (OR = 2.47). Depression, anxiety, and obsessive-compulsive symptoms were more severe in binge–purge AN and BN than in restrictive AN. BED and subthreshold BED impaired quality of life, while AN increased healthcare utilization. Stigma intensified isolation across subtypes. These findings support early screening, trauma-informed care, and culturally responsive services. Further longitudinal, community-based research is needed.

Keywords: Eating disorders, Adolescent health, Young adults, Anorexia nervosa, Bulimia nervosa, Binge-eating disorder, Psychiatric comorbidity

Abbreviations: AN: Anorexia Nervosa, **BED:** Binge-eating disorder, **BN:** Bulimia Nervosa, **AN-R:** Restricting Anorexia Nervosa, **DSM-5:** Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition**, ED:** Eating Disorder, **ARFID:** Avoidant Restrictive Food Intake Disorder

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1. Introduction

Feeding and eating disorders (EDs) are serious mental health conditions marked by persistent disturbances in eating behaviors and distressing thoughts or emotions related to food and body image. Although EDs were first clinically recognized in the 1970s, understanding of their complexity has grown considerably, with evolving diagnostic classifications over time. The text revision of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5-TR), currently categorizes anorexia nervosa (AN), bulimia nervosa (BN), binge-eating disorder (BED), avoidant/restrictive food intake disorder (ARFID), rumination disorder, and pica as the primary forms of EDs (APA, 2022).

Global surveillance data indicate that diagnosed ED cases have risen sharply among youth over the past decade, reflecting both increased detection and a genuine rise in incidence. Lifetime prevalence in Western countries is estimated at 0.16% for AN, 0.63% for BN, and 1.53% for BED (Keski-Rahkonen, 2021; Qian et al., 2022). A 2023 meta-analysis found that nearly 1% of children under 18 years met the criteria for an ED, with 0.6% diagnosed with AN and 0.1% with BN. Furthermore, disordered eating behaviors now affect as many as 1 in 5 individuals aged 11-25 years. Among females aged 5-30 years, lifetime prevalence rates of DSM-5-classified EDs range from 8.6% to 17.9%, compared with 2.2% to 4.1% among males (APA, 2022).

Each ED subtype follows a unique clinical trajectory. AN often begins between 10 and 20 years of age, peaks around 15 years, and is characterized by intense fear of weight gain, distorted body image, and severe dietary restriction. AN is predominantly identified in female populations and carries severe health consequences, including cardiac complications and bone depletion (Silén & Keski-Rahkonen, 2022; Wall et al., 2024). It is also the most medically severe eating disorder subtype, with the highest mortality rates and frequent psychiatric comorbidities (Fricke & Voderholzer, 2024). BN is a cyclical eating disorder that usually begins between 13 and 20 years of age and is characterized by binge-eating episodes followed by compensatory behaviors such as purging or excessive exercise. It may cause gastrointestinal damage, electrolyte imbalance, and dental erosion. BED, the most common ED worldwide, often develops between 12 and 24 years of age and is strongly associated with psychological distress, secondary metabolic disorders, and obesity (Keski-Rahkonen, 2021).

ARFID involves limited food variety or intake volume without body-image concerns, whereas other specified feeding or eating disorder (OSFED) is a DSM-5 category for individuals with serious eating-related symptoms and distress whose symptoms do not fully match the defined criteria for AN, BN, or BED (APA, 2022). Atypical anorexia and purging disorder are considered other specified EDs; they may affect up to 11.5% of young females and are often underdiagnosed (Silén & Keski-Rahkonen, 2022). Although other specified EDs are usually more common in females, gender patterns differ by subtype, and some presentations do not follow the usual female predominance. Recent research, including Hebebrand et al., suggests that AN and atypical AN may lie on a shared continuum, indicating that restrictive eating behaviors can occur at any body weight and in any gender and should not be overlooked (Hebebrand et al., 2024). However, neither ARFID nor OSFED is the main focus of this review; relevant comparative data are retained where available.

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The etiology of EDs is complex. Biological factors include genetic predisposition, neurochemical dysregulation, and early childhood weight changes (Liebman et al., 2020). ED risk is further aggravated by psychological factors such as low self-esteem, trauma, and perfectionism (Hambleton et al., 2022). Environmental conditions, including social-media exposure, internalization of thinness ideals, and culturally complex family processes, are now recognized as central to the development and maintenance of EDs. Although global awareness of EDs among young people is increasing, the evidence remains scattered across diagnostic types and developmental stages.

While awareness of EDs in youth is growing globally, existing research remains scattered across isolated diagnostic frameworks and age brackets. Most studies evaluate these conditions separately, creating a major gap in understanding the intersection of clinical, developmental, psychological, and systemic factors in AN, BN, and BED among youth. This paper addresses these empirical limitations by synthesizing recent post-DSM-5 findings on prevalence, BMI trends, age of onset, symptom persistence, psychiatric comorbidities, and barriers to care. The primary focus is on AN, BN, and BED, with comparative insights on atypical AN, other specified EDs, and ARFID included where relevant for contextual clarity.

2. Methodology

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 framework, which ensures transparency and reproducibility in study selection, data extraction, and reporting (Page et al., 2021). Because the included studies were observational and the review focused on exposures and outcomes rather than interventions, the Meta-analysis of Observational Studies in Epidemiology (MOOSE) checklist was applied to strengthen methodological rigor in reviewing epidemiological literature (Brooke et al., 2021). The review aimed to summarize quantitative and mixed-methods studies examining AN, BN, and BED, with comparative inclusion of atypical AN, ARFID, or other specified disorder presentations when directly analyzed against these three. The focus was on adolescents and young adults, specifically prevalence, BMI trajectories, age of onset, symptom duration, psychiatric comorbidities, and barriers to care. Studies were included if they used recognized diagnostic systems, such as DSM-5 or ICD-10, or validated tools for assessing ED symptoms, body image, or psychiatric comorbidity. This approach ensured diagnostic consistency without restricting inclusion to a single instrument.

2.1 Eligibility Criteria

The eligibility criteria for this review were developed using PRISMA 2020 and MOOSE guidelines to ensure a transparent and consistent selection process. Journal impact factor was not used as a restriction. Instead, publications in peer-reviewed, indexed journals were required to ensure that included studies met basic standards of methodological transparency, ethical review, and reporting quality. Studies with at least 50 participants were selected to improve the reliability of the findings, with particular focus on designs comparing two or more eating-disorder groups, as this enabled meaningful synthesis across diagnostic subtypes.

To capture the developmental progression of EDs, participants up to 30 years of age were included. Although this upper limit reflected the transition from adolescence into emerging adulthood, most included studies (14 out of 18) focused on adolescents under 18 years. Therefore, adolescent-only findings were examined separately where possible to identify key developmental patterns. The search window (2016-2025) was chosen to ensure that included studies reflected DSM-5 diagnostic definitions and to reduce variability in disorder classification. Detailed inclusion and exclusion criteria are summarized in Table 1.

Table 1. Eligibility criteria of systematic review

Criteria	Inclusion	Exclusion
Publication Type	Peer-reviewed journal articles	Reviews, meta-analyses, editorials, commentaries, conference abstracts, books, and non-peer-reviewed sources
Study Objective	Investigate the impact of AN, BN, and BED on adolescents and young adults, with explicit focus on prevalence, symptom presentation, weight/BMI trajectories, psychiatric comorbidities, and access-to-care disparities. Comparative findings involving other specified disorders, atypical AN, purging disorder, or ARFID were included only when reported within eligible studies.	Studies focusing exclusively on non-ED conditions, prevention programs without clinical outcomes, or unrelated psychiatric disorders.
Design / Methodology	Quantitative or mixed-methods designs with standardized diagnostic criteria (e.g., DSM-5 or ICD-10) or validated screening tools.	Qualitative studies, scoping or systematic reviews, case reports, clinical trials, randomized controlled trials, studies without clear diagnostic or screening criteria.
Time Frame	Published between 2016 and 2025	Published before 2016
Language	English	Non-English publications
Population	Adolescents and young adults up to 30 years	Studies focused exclusively on adults >30 years
Sample Size	≥50 participants	<50 participants

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2.2 Search Strategy

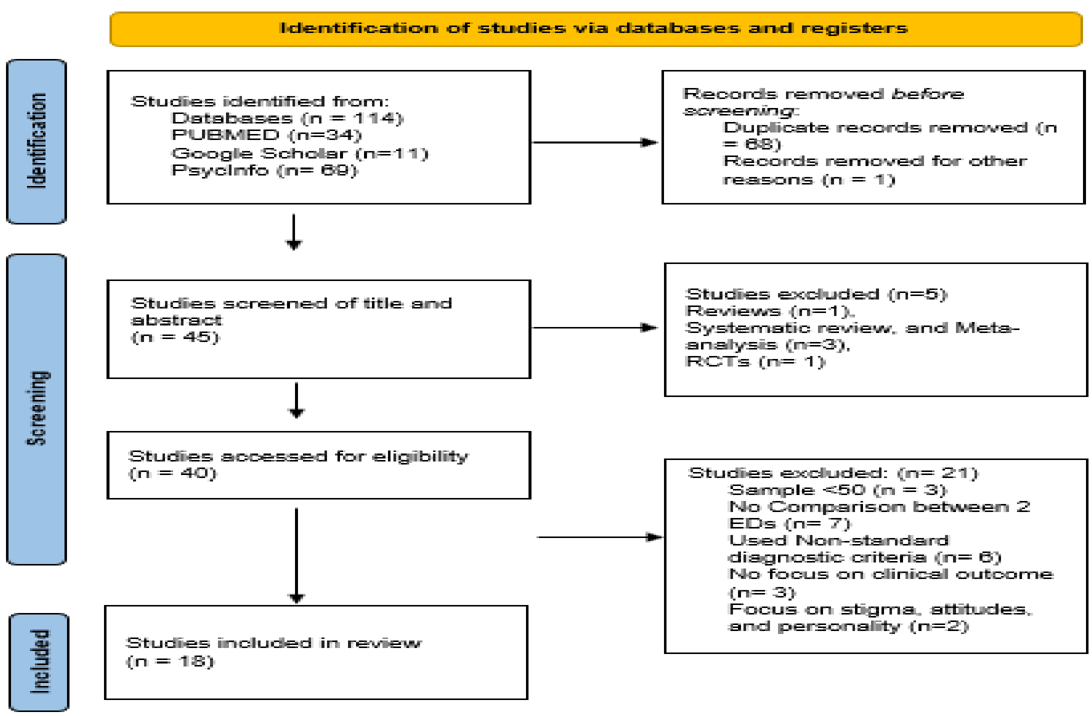
The literature search was conducted in three databases: PubMed, Google Scholar, and PsycINFO. Medical Subject Headings (MeSH) and keyword combinations such as "adolescent," "eating disorder," "anorexia nervosa," "bulimia nervosa," "binge eating disorder," "comparison," "comparative study," and "young adults" were used to facilitate the search. Boolean operators and title-specific search tags (e.g., allintitle:) were applied to capture relevant variations. Citations were managed in EndNote, which was used to identify and remove duplicates.

2.3 Study Selection

Titles and abstracts were independently screened by two reviewers against the eligibility criteria. Full texts of potentially eligible articles were retrieved and reviewed in duplicate by both reviewers. Disagreements were resolved by re-examining the original articles and reaching consensus through discussion. The selection process is illustrated in the PRISMA flow diagram (Figure 1).

Figure 1. PRISMA flow chart for the screening and identification of studies

Figure 1. PRISMA 2020 flow diagram showing study identification, screening, eligibility, and inclusion. Database searches returned 114 records; 68 duplicates were removed and 1 record was removed for other reasons, leaving 45 records for title/abstract screening. Forty full-text articles were assessed for eligibility, and 18 studies met the inclusion criteria and were included in the review.



2.4 Data Extraction

A structured data-extraction form was developed to collect variables such as sample size, geographic location, and population characteristics (age and sex), as presented in Table 2. Extracted data summarized the diagnostic tools, psychometric instruments, types of EDs compared, prevalence rates, BMI trajectories, comorbid conditions, and treatment-access details. One reviewer conducted data extraction independently, and the extracted information was fully cross-checked and independently verified by a second reviewer across the entire dataset. Discrepancies were resolved by re-examining the original articles. Studies with missing or insufficient data were excluded during the final review stage. Extended characteristics of included studies are presented in Table S1 in the supplementary data.

Table 2. Summary of included studies and key findings

Study (Year)	Country	Sample Size (Age)	Disorders Examined	Key Clinical Findings	Diagnostic Basis
Al Hourani et al. (2020)	Jordan	n = 1,001 (Mean: 16.8 years)	AN, BN, BED	BED was more common among individuals with dysglycaemia; older adolescents exhibited higher BN rates; sedentary behavior was inversely related to BN and BED risk.	EDDS
Andersen et al. (2023)	Denmark	n = 2,509 (16-17 years)	BED, Subthreshold BED	Subthreshold BED prevalence was 2.7%. Both full-syndrome BED and subthreshold BED were linked with depression, suicidality, and reduced quality of life.	DSM-5
Caspi et al. (2017)	Israel	n = 105 (15-25 years)	AN-Restrictive, AN-Binge/Purge, BN	Body-image disturbances differ significantly across ED phenotypes. Computerized screening tools distinguish ED	CDRS, CABI, EDI-2

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				patients from healthy controls effectively.	
Couturier et al. (2023)	Canada	n = 1,449 (5-17 years)	AN, BN, BED	Higher mental-health service utilization was observed for all EDs; patients with AN had the highest inpatient and clinical visit rates.	Clinical/administrative diagnoses
Curzio et al. (2018)	Italy	n = 419 (7-18 years)	AN, BN	AN linked with dietary restriction whereas BN is linked with bulimic behavior. Purging subtypes are tied to affective/interpersonal issues; BED presentations appeared independent.	Clinical subtype classification
Lewis et al. (2019)	Israel	n = 209 (<18 years)	AN-restrictive, AN-binge/purge, BN	Binge-purge AN showed higher obsessive-compulsive disorder, depression, and ED severity at admission. All groups showed clinical improvement by discharge.	DSM diagnoses + Y-BOCS, BDI, EAT-26
Lie et al. (2021)	Norway	n = 890 (Mean: ~29 years)	Restricting vs. binge/purge spectrum	Trauma, including sexual and emotional abuse, was more common in binge-purge subtypes; exposure to multiple stressful life events (SLEs) increased the odds of EDs.	Self-report ED subtype + SLEs

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Murakami et al. (2016)	USA	n = 318 (Mean: 21.6 years)	AN, BN, BED	AN and BN were the most stigmatized, BED was moderately stigmatized, and obesity was the least stigmatized; EDs were perceived as highly impairing.	Vignette-based
Reinehr et al. (2019)	Germany	n = 31,556 (12-18 years)	AN, BN, BED	EDs in Type 1 diabetes were linked to delayed puberty, insulin pump nonuse, higher HbA1c, diabetic ketoacidosis, and hypoglycemia; markers appeared soon after T1DM onset.	DPV registry diagnoses
Vázquez-Velázquez et al. (2017)	Mexico	n = 133 (15-19 years)	AN, BN, BED	Strongest behavioral correlations were observed in BED dyads; AN dyads showed inverse correlation, whereas BN showed moderate correlation, supporting disorder-specific behavioral transmission patterns.	Clinical diagnoses
Diaz Muñoz (2021)	Colombia	n = 1,545 (Mean: 19.2 years)	AN, BN	Overall, 27.6% of the group was classified as being at risk for an ED, with higher prevalence in females. Risk was directly linked to unhealthy dietary habits and appearance-driven exercise.	SCOFF, IPAQ

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Sandri et al. (2024)	Spain	n = 9,692 (18-30 years)	AN, BN	AN prevalence was 1.04%, and BN prevalence was 0.73%. EDs were linked with poorer sleep, energy-drink and coffee use, and higher overall rates in females.	NutSo-HH Scale
Yilmaz et al. (2019)	UK	n = 1,502 (birth-18 years)	AN, BN, BED	AN trajectory diverged below controls by age 4 years. Conversely, BN, BED, and purging-disorder trajectories rose above controls by age 6 years. Early BMI divergence may signal early ED risk.	ALSPAC + ED diagnoses at 14,16,18
Yamamiya & Stice (2024)	USA	n = 492 (11-15 years)	AN, BN, BED	BN, BED, and purging disorder were predicted by thin-ideal pressure and low parental support; AN was predicted only by low BMI.	Annual diagnostic interviews
O'Connor et al. (2016)	Ireland	n = 290 (15-19 years)	AN, BN, BED	EDs are more stigmatized than depression/diabetes; BED is viewed as a lack of self-control.	Vignette-based
Zanna et al. (2021)	Italy	n = 346 (10-15 years)	AN-Restrictive, Atypical AN, ARFID	ARFID exhibited a distinct symptom profile and comorbidities compared with restrictive conditions, although shared vulnerabilities	DSM-5 + psychometrics

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				existed across all groups.	
Ernst et al. (2017)	Germany	n = 1,654 (12-15 years)	AN, BN	DSM-5 criteria increased the prevalence of detection and improved clinical diagnostic differentiation.	DSM-IV vs. DSM-5
Burt et al. (2020)	Australia	5,068 (14-15 years)	AN, BN, other specified EDs	OSFED showed higher prevalence in First Nations youth. Across all diagnostic groups, EDs were linked to lower psychosocial quality of life.	DSM-5

2.5 Quality Assessment

Risk of bias was systematically assessed to evaluate the methodological quality of the included evidence using the Newcastle-Ottawa Scale (NOS). Two independent reviewers performed the quality appraisals across selection, comparability, and outcome/exposure assessment. Any disagreements were resolved through discussion until consensus was reached.

3. Results

3.1 Study Selection and Characteristics

The systematic search strategy identified 114 articles published between 2016 and 2025. After duplicates were removed and studies that did not meet the inclusion criteria were excluded, 18 studies were included in the final review (Fig. 1). The studies represented a broad geographical scope across 14 countries: USA (2 studies, n = 810), Italy (2 studies, n = 765), Israel (2 studies, n = 314), Denmark (1 study, n = 2,509), Canada (1 study, n = 1,449), Norway (1 study, n = 890), Germany (2 studies, n = 33,210), Mexico (1 study, n = 133), Colombia (1 study, n = 1,545), Spain (1 study, n = 9,692), the UK (1 study, n = 1,502), Ireland (1 study, n = 290), Jordan (1 study, n = 1,001), and Australia (1 study, n = 5,068). Most studies were conducted in high-income countries, except those from Jordan and Colombia, which represented middle-income settings and contributed to broader global representation.

The total sample size across the studies was 59,178 individuals (mean age 16.96 years; range: birth to <30 years). Based on available reporting, 77.1% of participants were female and 6.5% were male. In some studies, sex or gender was not reported or was unspecified for the remaining

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participants. Included quantitative studies comprised cohort (n = 5), cross-sectional (n = 6), case-control (n = 3), longitudinal observational (n = 2), and experimental/vignette-based designs (n = 2). Most studies were conducted in clinical settings (n = 12, 66.7%), followed by university populations (n = 5, 27.8%) and one school-based setting (n = 1, 5.6%). The reviewed studies primarily compared AN, BN, and BED with respect to prevalence, psychiatric comorbidities, age of onset, early BMI trajectories, familial and environmental risk factors, and treatment barriers; studies that directly compared atypical AN, ARFID, or OSFED presentations were also retained. Two studies employed longitudinal designs (15, 16), providing insight into predictive factors and early markers of ED onset.

The included studies covered a broad age range, although most focused on adolescents (n = 14, 77.8%), with fewer examining young adults (n = 4, 22.2%). Sample sizes varied widely, from 105 participants (17) to 31,556 participants (18). All included studies used recognized diagnostic systems and validated psychometric or clinical assessment tools appropriate to their design. In terms of diagnostic focus, seven studies examined AN (38.8%), eight focused on BN (44.4%), and five focused on BED (27.7%). Some studies included mixed samples of adolescents and young adults or individuals with subthreshold ED symptoms. Most studies examined how ED behaviors were linked to mental-health concerns such as depression, anxiety, and suicidal ideation.

The Newcastle-Ottawa Scale (NOS) was used for risk-of-bias assessment and evaluates three domains: selection (up to 4 stars), comparability (up to 2 stars), and outcome/exposure (up to 3 stars). Studies scoring ≥7 stars were considered low risk, 5-6 stars moderate risk, and <5 stars high risk. Of the 18 studies, 7 were rated as low risk, 10 as moderate risk, and 1 as high risk. Most studies were conducted in well-established clinical settings and used validated diagnostic methods, although some relied on subjective or self-reported data, increasing the likelihood of measurement and recall bias. A full breakdown of risk-of-bias ratings is provided in Table S2 in the supplementary material.

3.2 Clinical Differences

The synthesized findings are organized according to four primary analytical objectives: (1) clinical and behavioral characteristics, (2) developmental trajectories, (3) psychological and familial correlates, and (4) systemic disparities in access to care.

3.2.1 Restrictive Control and Cognitive Rigidity

Restrictive eating in AN, particularly in the restricting subtype (AN-R), is strongly linked to rigid thinking, inflexible food-related rules, and overcontrolled self-regulation. These traits manifest not only in behavior but also in perceptual processing. Adolescents with AN showed greater body-size overestimation on computerized tests than controls (Caspi et al., 2017). These distortions reinforce restrictive behavior by maintaining fear of weight gain even at low BMI. Developmental evidence suggests that restrictive tendencies may begin early. Children who later developed AN had already diverged from growth norms in early childhood, with significantly lower BMI by age 4 years in girls and by age 2 years in boys (Yilmaz et al., 2019). This pattern indicates that AN-R may reflect an overcontrolled self-regulation phenotype characterized by cognitive rigidity, fear of weight gain, and distorted body image.

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3.2.2 Binge and Compensatory Behaviors

Unlike restrictive control, BN and BED are characterized by loss of control over eating and difficulty regulating emotions. BN involves repeated binge-eating episodes followed by compensatory behaviors such as purging or fasting, whereas BED involves binge eating without compensatory behaviors (Curzio et al., 2018). Symptom severity was more apparent in purging-type disorders (BN and binge-purge AN) than in AN-R, leading to greater distress, impulsive eating, and behavioral instability (Lewis et al., 2019). BED was also strongly linked with affect-regulation difficulties, negative mood, and higher metabolic risk. In both full-threshold and subthreshold BED, binge-episode frequency was the strongest predictor of psychological distress (Andersen et al., 2023; Vázquez-Velázquez et al., 2017). Medical vulnerabilities further shaped risk. Adolescents with dysglycaemia demonstrated notably elevated BED rates (Al Hourani et al., 2020), suggesting a reciprocal link between metabolic disruption and binge-eating behavior. Sociocultural pressures also contributed to risk: thin-ideal internalization and appearance-focused social comparison predicted later bulimic and binge-eating symptoms (Yamamiya & Stice, 2024). Trauma exposure was also identified as a major risk factor, as adolescents who experienced sexual or emotional abuse were more likely to develop later bulimic or binge-eating behaviors (Lie et al., 2021). This association aligns with affect-regulation models, in which disordered eating functions as a maladaptive coping strategy for stress. Trauma-linked explanations are discussed in Section 3.2.5.3.

3.2.3 Shared and Distinct Psychopathology Across Subtypes

Although EDs share several core features, including body-image disturbance, psychological distress, and functional impairment, each subtype expresses these symptoms differently. Comparative findings summarized in Table 3 show that BN and binge-purge AN exhibit the most severe body-image distortion and preoccupation (Caspi et al., 2017). These groups also tend to cluster on affective instability and interpersonal difficulties, whereas restrictive AN cluster on cognitive rigidity and overcontrolled self-regulation (Curzio et al., 2018). BED differs by being closely linked to emotional dysregulation and impulsive eating, with less emphasis on weight-management behaviors. Behavioral patterns also differed. Individuals with AN/BN consumed more energy drinks (34% higher) and fewer ultra-processed foods (22% lower) than healthy controls (Castillo & Weiselberg, 2017). These compensatory consumption patterns may reflect attempts to regulate energy balance or perceived control over intake. Overall, these findings suggest a spectrum of self-regulatory dysfunction: AN-R is overcontrolled, BED is under controlled, and BN oscillates between the two.

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Table 3. Comparative Clinical and Psychological Features by Disorder Subtype

Domain	AN / Atypical AN	BN	BED / SBED	References
Mean age of onset	10-20 years (Earliest among EDs)	13-20 years	12-24 years	Castillo & Weiselberg (2017) and Keski-Rahkonen (2021)
Core behavioral pattern	Severe restriction (AN-R) or restriction with binge-purge behavior (AN-BP)	Recurrent binge + compensatory purging	Recurrent binge without compensation	Qian et al., (2022) and Salari et al. (2025)
BMI trajectory	Low BMI by age (2-4 years)	Higher BMI by age (6 years)	High BMI and metabolic risk	Ghazzawi et al. (2023)
Psychiatric comorbidities	Anxiety > Controls; obsessive-compulsive traits high (Y-BOCS = 20-28)	Depression and anxiety elevated	Highest depression scores (mean = 28)	Qian et al. (2022) and Salari et al. (2025)
Trauma associations	Moderate; emotional abuse link in binge-purge AN	Strong link with sexual/emotional abuse (OR ≈ 2.5)	Strong link with trauma and affect-regulation issues	Qian et al. (2022)
Personality traits	Cognitive rigidity, perfectionism	Impulsivity, emotional instability	Negative emotionality	Castillo & Weiselberg (2017)
Quality of life	Impaired physical health and social quality of life	Greater psychological distress	Most severe quality of life impairment	Keski-Rahkonen (2021)
Care access / stigma	High hospitalization but timely diagnosis	Under-recognized in males and minority groups	Least likely to seek help due to stigma	Ghazzawi et al. (2023) and Silén & Keski-Rahkonen (2022)

3.2.4 Developmental and Longitudinal Patterns

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Developmental evidence highlights adolescence as a critical period for the emergence and progression of EDs. Restrictive disorders, especially AN, appeared earliest. Across populations, children who later developed AN showed lower BMI trajectory from early childhood. Girls had lower BMI than controls by age 4 years (mean BMI = 16.5 kg/m²), whereas boys showed lower BMI by age 2 years (mean BMI = 17.0 kg/m²) (Yilmaz et al., 2019). For pediatric samples, BMI z-scores were used to adjust for normal growth, making groups comparable by age. AN also showed greater chronicity, with symptoms lasting more than two years in roughly 60% of cases, reinforcing its early-onset and persistent course. In contrast, BN, BED, and purging disorder symptoms typically appeared around age 9.5 years and were associated with higher childhood BMI profiles (mean BMI = 22.5 kg/m²) (Yilmaz et al., 2019). Longitudinal evidence further supports adolescence as a high-risk period. A prospective cohort of girls aged 11-15 years found early risk factors for later AN, BN, and BED, although these disorders followed distinct developmental patterns (Yamamiya & Stice, 2024). Adolescents aged ≥14 years showed more bulimic behavior than younger adolescents (Al Hourani et al., 2020), suggesting a developmental shift toward compensatory pathology, impulsivity, and body dissatisfaction. These vulnerabilities may be aggravated by chronic disease. Reinehr et al. found that ED symptoms appeared soon after Type 1 diabetes mellitus (T1DM) diagnosis and posed ongoing challenges for disease management (Reinehr et al., 2019). This overlap supports closer integration of medical and psychological care, as physiological monitoring may intensify appearance-related concerns. EDs may also persist into young adulthood. Diaz Muñoz et al. reported an ED-risk prevalence of 27.6% among undergraduates aged 18-30 years (Díaz Muñoz, 2021), while Sandri et al. reported AN and BN prevalences of 1.04% and 0.73%, respectively, in a comparable young-adult cohort (Castillo and Weiselberg 2017). Overall, developmental data suggest that restrictive pathology may originate early through biologically driven patterns, whereas binge-spectrum disorders appear more strongly shaped by emotional, psychosocial, and environmental risk factors.

3.2.5 Psychiatric Comorbidities in Eating Disorders: Patterns and Implications

3.2.5.1 Depression and Suicidality

Higher levels of depressive symptoms have been reported in binge-type disorders. In community samples, BED (1.1%) and subthreshold BED (2.7%) were associated with depression, suicidal ideation, and reduced quality of life. The highest depression scores were observed in BED (mean = 28.1), followed by other EDs (mean = 24.5) and controls (mean = 14.3) (Andersen et al., 2023). BN and BED onset were also associated with negative emotionality (beta = 0.35), which is closely related to depression and anxiety (Yamamiya & Stice, 2024). These results indicate that mood dysregulation does not simply arise from disordered eating; it may also help maintain it. Depression and disordered eating often co-occur, which may help explain why individuals with binge-purge behaviors face a higher risk of suicide.

3.2.5.2 Anxiety and Obsessive-Compulsive Features

Anxiety differs across subtypes and reflects underlying patterns of control. Patients with binge-purge AN/BN recorded higher anxiety scores at treatment admission and more intrusive thoughts,

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consistent with emotional instability (Lewis et al., 2019). Anxiety was also elevated in restrictive groups. Both AN-R and atypical AN had higher anxiety than ARFID (Zanna et al., 2021), whereas BED was associated with a high level of general anxiety risk (risk ratio = 3.7) (Andersen et al., 2023). Overall, individuals with EDs showed higher total anxiety on the State-Trait Anxiety Inventory (STAI mean = 45.3 vs. controls = 35.6) (Vázquez-Velázquez et al., 2017). Obsessive-compulsive features followed a parallel pattern. Yale-Brown Obsessive Compulsive Scale scores indicated that binge-purge AN (Y-BOCS = 28.5) involved more severe obsessive-compulsive symptoms than AN-R (Y-BOCS = 19.8) (Lewis et al., 2019). Restrictive presentations, including AN-R and atypical AN, also carried obsessive-compulsive tendencies, consistent with evidence of rigid, overcontrolled cognitive style s (Zanna et al., 2021). Comorbid anxiety disorders were also common in metabolic subgroups. In Jordanian adolescents with dysglycaemia, anxiety significantly increased the risk of BN and BED, whereas sedentary behavior unexpectedly reduced risk, possibly reflecting anxiety-driven avoidance (Al Hourani et al., 2020). Separation anxiety symptoms were particularly prominent in ARFID, distinguishing it from weight-focused restrictive disorder s (Zanna et al., 2021). These comorbidity patterns mirror broader regulatory profiles: overcontrol in restrictive disorders and anxiety-driven dysregulation in binge-purge forms.

3.2.5.3 Trauma and Stressful Life Events

Traumatic exposure was associated with binge-purge presentations. Lie et al. reported that 93% of female ED patients had experienced at least one stressful life event. Those exposed to sexual assault had more than twice the odds of developing binge-purge AN, BN, or BED (odds ratio = 2.24) (Lie et al., 2021). Specific events such as rape (OR = 3.58), other sexual assault (OR = 2.96), and emotional abuse (OR = 2.15) carried even higher risk. Risk continued to rise as the number of traumatic events increased. Patterns from other samples also support this association. In an Italian multisite study, binge-purge presentations (binge-purge AN, purging BN, and non-purging BN) clustered on dimensions related to affective distress and interpersonal difficulties, whereas restrictive AN and ARFID did not show these trauma-related patterns (Curzio et al., 2018). Intergenerational effects were also noted. Mothers of daughters with BN and BED reported more past traumatic experiences than mothers of daughters with AN (Vázquez-Velázquez et al., 2017). This evidence suggests that sexual, emotional, and cumulative trauma are strongly and selectively related to binge-eating and purging-type EDs. In contrast, restrictive AN and ARFID appear less strongly related to trauma exposure in the available data.

3.2.5.4 Personality Traits and Familial Dynamics

Personality traits and family dynamics also followed subtype-specific patterns. Restrictive presentations tended to share a similar profile. In an Italian study, individuals with AN-R, atypical AN, and ARFID showed similar elevations in interpersonal insecurity, emotional dysregulation, and difficulty interpreting internal cues (Zanna et al., 2021). In contrast, binge-purge presentations were consistently linked with higher clinical perfectionism, mood instability, and interpersonal difficulties (Curzio et al., 2018; Lewis et al., 2019). Family patterns also differed by subtype. Family modelling was most noticeable in BED, especially between mothers and daughters. BN showed a moderate level of symptom similarity within families, whereas AN showed the weakest

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family resemblance, suggesting a stronger biological contribution (Vázquez-Velázquez et al., 2017).

3.3 Multifaceted Impact of Eating Disorders on Quality of Life

Quality-of-life impairment is common across ED subtypes and arises from the combined psychological, social, and physical burdens of disordered eating. Adolescents with full-threshold and subthreshold BED reported the greatest psychosocial distress (Andersen et al., 2023). Among First Nations Australian youth, poor psychosocial quality of life was most pronounced in other specified EDs (Burt et al., 2020). Similar patterns were observed in non-Indigenous adolescents, confirming that ED-related distress was not culturally specific c (Burt et al., 2020). Physical health consequences also contributed significantly. In young adults, Sandri et al. found that AN and BN were associated with poorer sleep quality, shorter sleep duration, and increased energy-drink consumption (Sandri et al., 2024). In adolescents with T1DM, ED symptoms were associated with poorer metabolic control, including increased HbA1c and higher rates of diabetic ketoacidosis and hypoglycemia, leading to physical-health deterioration (Reinehr et al., 2019).

The emotional and social burden of EDs aggravates these quality-of-life impairments. Greater symptom severity, particularly obsessive-compulsive symptoms in binge-purge AN and depression in BN/BED, was linked to poorer mental well-being and self-esteem (Lewis et al., 2019). The emotional impact of these disorders did not depend solely on symptom intensity. Psychosocial factors, particularly low parental support and negative emotionality, amplified these impairments (Yamamiya & Stice, 2024). Social context further shaped quality-of-life outcomes. Stigma played an additional role: AN and BN were viewed as more blameworthy and less legitimate than obesity or BED, which may deter early help seeking and worsen distress (Murakami et al., 2016). Family dynamics, especially in BED, contributed to symptom maintenance and daily functional challenges (Vázquez-Velázquez et al., 2017). These results emphasize the need for treatment approaches that target not only symptom reduction but also emotional regulation, metabolic stability, and social reintegration.

3.4 Access to Care and Treatment Outcomes

3.4.1 Healthcare Disparities and Access Inequities

Access to ED care differed widely across groups and was shaped by clinical severity as well as social and structural barriers. Across settings, restrictive and purging presentations produced more hospitalizations and mental-health visits than ARFID or BED (Couturier et al., 2023). These trends suggest greater clinical severity, but they also reveal problems in continuity of outpatient care. A similar breakdown in continuity appeared in chronic medical populations, where adolescents with T1DM did not receive frequent ED screening despite clear metabolic risks and poorer health outcomes (Reinehr et al., 2019). Similar gaps were evident among dysglycaemic youth, who showed increased BED rates but lacked formalized referral pathways (Al Hourani et al., 2020). Beyond chronic medical populations, weight-related and cultural disparities further delayed detection. These delays were common among higher-weight adolescents, who were often advised to lose weight rather than evaluated for ED symptoms (Murakami et al., 2016), and among First

Nations adolescents, who reported poorer psychosocial well-being yet had fewer opportunities for specialist treatment (Burt et al., 2020). Gender differences were also present. ED symptoms in boys were frequently overlooked because most diagnostic systems and screening practices implicitly favored female presentations (Díaz Muñoz, 2021; Sandri et al., 2024). Sexual- and gender-diverse adolescents were underrepresented in most studies, which represents a significant evidence gap. Overall, these differences show that early detection is often limited not by symptoms themselves but by social and structural factors. Improving primary-care screening, reducing stigma, and addressing weight- and culture-related biases are crucial for equitable access

3.4.2 Treatment Outcomes and Novel Approaches

Responses to inpatient treatment differed by subtype. Adolescents with binge-purge AN/BN showed greater improvements in obsessive-compulsive symptoms, depression, and anxiety between admission and discharge than those with restricting AN, although changes in eating-related measures were similar (Lewis et al., 2019). This trend indicates that binge-purge presentations may respond more strongly to standard multimodal inpatient care. In contrast, restrictive presentations often involve more rigid thinking and behavioral inflexibility, indicating a need for interventions that specifically target these traits. Alongside these treatment insights, methodological advances are improving assessment and monitoring. For instance, computerized body-image instruments distinguished ED subtypes from controls more accurately than conventional silhouette measures (Caspi et al., 2017). Such body-image assessment may be valuable for early detection and for monitoring perceptual distortions without requiring extensive additional resources. Despite these advances, ED screening remains inconsistent. Trauma history, which is strongly associated with binge-purge presentations, is rarely included in standard evaluations (Lie et al., 2021). Restrictive and partial-syndrome presentations often receive insufficient attention, and ARFID shares emotional and developmental vulnerabilities with AN but remains underserved in routine care (Zanna et al., 2021; Couturier et al., 2023). Overall, treatment outcomes indicate that standard approaches benefit some subtypes more than others. Greater integration between medical and mental-health services, trauma-informed evaluation, and subtype-specific therapeutic models is necessary to improve treatment effectiveness across the spectrum.

4. Discussion

Across the literature examined, three cross-cutting themes emerged. First, eating disorders in adolescence follow different developmental pathways. Restrictive symptoms tend to emerge earlier, often preceded by lower BMI trajectories and over-controlled behavioral styles, and typically persist longer. In contrast, later-onset binge-purge symptoms were more strongly tied to emotional dysregulation and trauma exposure. Second, shared psychopathology, including anxiety, depression, and body-image disturbance, was present across all subtypes. However, the expression of these symptoms differed, with restrictive disorders marked by cognitive rigidity and compulsive control, and binge-purge disorders shaped by impulsivity, affect-regulation difficulties, and interpersonal strain. Third, systemic and structural inequities shape access to care, particularly for adolescents in higher-weight bodies, boys, gender-diverse youth, youth of First Nations, and those with chronic medical conditions such as T1DM. Weight stigma, diagnostic delays, and poor coordination between medical and mental-health services consistently

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contributed to under-recognition, even when metabolic risk or psychosocial impairment was apparent. These patterns highlight that adolescent EDs unfold within "uneven social systems," where recognition and treatment depend as much on structural context as on clinical presentation.

The existing evidence aligns with reports of increasing ED prevalence among adolescents worldwide (Galmiche et al., 2019). It supports prior evidence linking restrictive control with anxiety-related behaviors (Fitzgerald et al., 2021) and the central role of sociocultural and familial influences in BN and BED (Sim et al., 2024). The developmental sequencing observed in this review, early BMI divergence in AN and later emergence of binge-purge behaviors, aligns with prospective work showing that biological and psychosocial vulnerabilities likely unfold at different stages of growth (Rohde et al., 2015). The included studies also highlight the importance of recognizing restrictive pathology in normative-weight adolescents. Although atypical AN was not a primary focus of the comparative studies included in this review, the broader evidence base emphasizes its clinical relevance. The findings of Zanna et al. (2021), along with recent conceptual work by Hebebrand et al. (2024), support the idea that AN and atypical AN share core restrictive psychopathology and likely fall along a single diagnostic spectrum. The limited representation of atypical AN in current comparative studies reflects gaps in the available research rather than a lack of clinical importance.

The primary strength of this paper is its systematic synthesis of AN, BN, and BED across prevalence, developmental patterns, psychiatric comorbidities, and care disparities. The inclusion of diverse study designs and international samples strengthens generalizability. Longitudinal and cohort data also provide useful insight into early risk factors and symptom development. Nevertheless, several limitations should be considered. The search may have excluded valuable non-English literature because only English-language publications were included. Some studies used self-report or secondary data, increasing the risk of measurement bias. Randomized controlled trials were not included, limiting causal inference. Not all samples reported sex/gender details, restricting gender-based analysis. Finally, comparative work involving atypical AN, ARFID, and gender-diverse youth remains underrepresented, highlighting important gaps in the evidence base. Despite these limitations, convergent trends across independent datasets strengthen the validity of the findings.

The findings demonstrate the need for earlier diagnosis and intervention that considers the developmental and psychological needs of young people. Routine screening of at-risk adolescents in schools, universities, and primary-care settings may help prevent symptom progression. Treatment should not focus only on eating symptoms; it should also address comorbid anxiety, depression, trauma history, and sociocultural pressures. Interventions that improve emotion regulation and reduce perfectionism may help differentiate and treat restrictive and binge-purge presentations. For sustained recovery, care must include consistent, coordinated follow-up that integrates family support with trauma-informed approaches.

Future studies should prioritize longitudinal, developmentally sensitive designs to examine the interplay among biological, emotional, and environmental factors across childhood, adolescence, and emerging adulthood. Atypical and weight-diverse restrictive presentations should also be studied to avoid reinforcing weight-based diagnostic bias. Including more male, gender-diverse, and minority youth is crucial for fair and reliable evidence. In clinical practice, early diagnosis, integrated medical-mental health pathways, and subtype-specific interventions may improve outcomes. Ultimately, culturally competent systems that recognize both psychological vulnerabilities and structural barriers are required to reduce the burden of EDs in young populations.

5. Conclusion

This paper summarizes recent evidence on how clinical, developmental, and systemic factors influence the phenotypic presentation and clinical course of eating disorders (EDs). Binge-purge profiles were more strongly correlated with trauma exposure, emotional dysregulation, and psychiatric comorbidities than restrictive phenotypes, whereas restrictive disorders showed greater medical severity and earlier onset. Early BMI differences and behavioral patterns suggest that risk may begin long before symptoms become obvious. Across clinical settings, significant disparities in detection and treatment were evident among higher-weight youth, boys, gender-diverse adolescents, and young people from racial or lower-income backgrounds. Weight stigma, limited screening, and poor coordination between medical and psychological services contributed to delayed care. These findings underscore the need for developmentally sensitive assessment, early and equitable identification, and integrated, culturally responsive care pathways. Improving continuity across outpatient, inpatient, and school-based health systems and expanding access to specialized services remain essential for reducing the burden of EDs in young populations.

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Author contributions

Conceptualization: Hafsa Naseer and Muhammad Tauseef Sultan; **writing - original draft:** Ahmad Mujtaba Noman and Zainab Ahmed; **writing - review and editing:** Hassan Raza and Muhammad Abdul Rahim; **draft preparation:** Shehnshah Zafar and Sabeen Zahra; **validation and visualization:** Hafsa Naseer, Ahmad Mujtaba Noman, and Hassan Raza. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Ethical statement / Clinical trial registration number / Informed consent

Not applicable.

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Note. Detailed study-level characteristics are provided in Supplementary Appendix A, including Table S1 (Characteristics of Included Studies) and Table S2 (Risk of Bias Assessment Using the Newcastle-Ottawa Scale).

Table 1. Characteristics of included studies

Study	Country	Sample Size	Age Range	Disorders Compared	Key Findings	Diagnostic Criteria
Al Hourani et al. (2020)	Jordan	1,001 adolescents: 497 with dysglycaemia (325 females, 65.4%; 172 males, 34.6%) and 504 controls (329 females, 65.3%; 175 males, 34.7%)	Mean age (16.8 ± 4.3)	AN, BN, BED, Other Eating Disorders (e.g., atypical anorexia, purging disorder)	Higher prevalence of BED in dysglycaemic adolescents (11.9% vs. 5.8%). Older adolescents (≥14) had higher bulimia rates. Sedentary lifestyle inversely related to bulimia and binge eating.	Eating Disorder Diagnostic Scale (EDDS)
Andersen et al. (2023)	Denmark	2,509 participants; more girls were invited for follow-up, but the exact number was not reported	16-17 years; mean age 16.5 years (SD = 4.2 months)	BED vs. SBED	SBED prevalence: 2.7%. Both SBED and BED are associated with depressive symptoms, suicidal ideation, and impaired quality of life. No link found with non-suicidal self-injury. Symptom impact is similar regardless of frequency/duration.	DSM-5 criteria (approximated via survey items)
Caspi et al. (2017)	Israel	105 (22 AN-R, 22 AN-B/P, 20 BN, 41 controls)	Females aged 15-25 years	AN-R, AN-B/P, BN, Healthy Controls	Significant differences in body image disturbances among ED types using three	CDRS, CABI, EDI-2-Body Dissatisfaction Scale

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					assessment tools (CDRS, CABI, EDI-2-BD). CABI effectively distinguished ED patients from controls. Multimodal assessments enhance understanding of body image distortions.	
Couturier et al. (2023)	Canada	AN (n = 674; 605 females, 89.8%; 69 males, 10.2%); BN (n = 230; 222 females, 96.5%; 8 males, 3.5%); BED (n = 59; 47 females, 79.7%; 12 males, 20.3%); ARFID (n = 171; 113 females, 66.1%; 58 males, 33.9%); other EDs (n = 315; 283 females, 89.8%; 32 males, 10.2%); plus a matched general-population cohort (5:1)	5-17 years; mean ages: AN 14.47 ± 1.83, BN 15.12 ± 1.34, BED 14.19 ± 1.97, ARFID 12.12 ± 3.01, OSEDs 14.39 ± 1.99 years	AN, BN, BED, ARFID, Other Specified EDs	Mental health care utilization was higher for all subgroups compared to the general population; the AN subgroup had significantly higher hospitalization and mental health visit rates compared to ARFID	Diagnostic classification based on clinical diagnoses from health administrativ e data
Curzio et al. (2018)	Italy	419 patients (13.8% males; 86.2% females)	7-18 years (mean age 14.7 ± 2.14)	AN, BN, EDNOS (including AN-R, bulimic	Both disorder-specific and transdiagnostic mechanisms exist. AN group	Clinical diagnoses based on ED subtype classification

				purging/non-purging, BED)	associated with restraint, BN group with bulimic behavior; purging subtypes showed homogeneity tied to affective and interpersonal issues. EDNOS opposed to AN-R; BED appeared independent.	in clinical centers
Lewis et al. (2019)	Israel	209 female adolescent inpatients (94 AN-R, 67 AN-B/P, 48 BN)	Adolescents under 18 years (15-16 years)	AN-R, AN-B/P, BN	AN-B/P patients showed higher obsessive-compulsive symptoms (Y-BOCS), eating-disorder severity (YBC-EDS), and depression (BDI) at admission compared with AN-R and BN. All groups improved by discharge, with AN-B/P and BN showing greater improvement in obsessionality and anxiety than AN-R.	Diagnoses per DSM criteria for AN-R, AN-B/P, and BN; symptom severity assessed via Y-BOCS, YBC-EDS, EAT-26, BDI, and STAI
Lie et al. (2021)	Norway	890 total (495 with EDs, 395 controls) 95% females	Mean ~29 years (±9.8 EDs, ±11.7 controls)	ED subtypes along the restricting to binge-eating/purging spectrum.	Individuals with EDs reported significantly more stressful life events (SLEs) than controls (OR=2.47). Sexual abuse (rape, assault) and emotional abuse were especially associated with binge-	Self-reported lifetime history of EDs and SLEs; ED subtypes divided by restricting vs. binge/purging behaviors

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					eating/purging subtypes. Multiple SLE exposures increased the association strength.	
Murakami et al. (2016)	USA	318 undergraduates (73.6% female)	Mean age: 21.58 (SD = 3.97)	AN, BN, BED, obesity	Stigma levels differed by diagnosis but not by gender; AN and BN were most stigmatized, followed by BED, with obesity least stigmatized; EDs were perceived to involve more psychological impairment than obesity	Vignette-based experimental design (diagnoses described in vignettes)
Reinehr et al. (2019)	Germany (DPV cohort)	31,556 girls with T1DM, including 155 (0.49%) with AN, 85 (0.27%) with BN, 45 (0.14%) with BED, and 229 (0.73%) with EDNOS	>6 months to <23 years; females aged 12-18 years were considered vulnerable to EDs	AN (n = 155), BN (n = 85), BED (n = 45), EDNOS (n = 229)	EDs associated with late puberty, pump nonuse, higher HbA1c, increased DKA, and severe hypoglycemia; weight loss predicted AN. Celiac disease not associated. ED indicators appeared early after T1DM onset.	Clinical diagnosis via DPV registry; likely DSM-based (not explicitly stated)
Vázquez-Velázquez et al. (2017)	Mexico	133 dyads (30 AN, 30 BN, 19 BED, 54 control)	Young females aged 15-19 years; AN 18.2 ± 2.9 years, BN 19.1 ± 3.1 years, BED 22.3 ± 3.1	AN, BN, BED, Non-ED	Inverse correlations in AN dyad; moderate correlations in BN dyads; strongest positive correlations in BED dyads (especially in	Clinical diagnoses and psychological assessment tools.

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			years, and controls 19.1 ± 2.7 years		eating behavior); control dyads showed low but significant correlations. Disorder-specific analysis recommended for treatment implications.	
Diaz Muñoz (2021)	Colombia	1,545 65.7% female	Mean age: 19.2 years (±2.5)	Risk of AN and BN	27.6% of students were at risk. Risk was higher in females and associated with dietary habits (fat and light products intake), protein supplement use (protective), and motivations for physical activity (appearance-related increased risk).	SCOFF questionnaire, IPAQ (short form), multivariate logistic regression
Sandri et al. (2024)	Spain	9,692 young adults (1,835 males, 18.93%; 7,857 females, 81.07%)	18-30 years; male mean age 24.28 ± 3.40 years; female mean age 24.37 ± 3.35 years; BN subgroup mean age 23.79 ± 3.19 years	AN, BN	Prevalence: AN = 1.04%, BN = 0.73%. Higher rates in women, regardless of sociodemographic factors. Those with EDs consumed less fast/ultra-processed food but more coffee and energy drinks. Poorer sleep quality and duration were noted. No difference in sedentary behavior; physical activity	Diagnosis based on responses to the NutSo-HH Scale (valid and reliable tool); not a clinical diagnosis

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					increased in bulimia cases.	
Yilmaz et al. (2019)	United Kingdom	1,502 (AN: 243, BN: 69, BED: 114, PD: 133, Controls: 966) 58% female	Birth to 18 years	AN, BN, BED, PD	AN: BMI trajectories diverged below controls by age 4 years in girls and by age 2 years in boys. BN, BED, and PD trajectories diverged above controls before age 6 years. Early BMI patterns may indicate biological risk or early signs of EDs.	ED diagnoses assessed at ages 14, 16, and 18; BMI tracked from birth to 12.5 years; based on Avon Longitudinal Study of Parents and Children (ALSPAC)
Yamamiya & Stice (2024)	USA (Texas)	492 adolescent girls	11-15 years at baseline; followed for 8 years; mean age 13.02 years (SD = 0.73)	AN, BN, BED & PD	AN: Only low BMI predicted onset. BN: Predicted by pressure to be thin, thin-ideal internalization, body dissatisfaction, negative emotionality, low parent support, and modeling of eating pathology. BED: Same predictors as BN except body dissatisfaction. PD: Predicted by pressure to be thin, body dissatisfaction, dietary restraint, low parent support, modeling of eating pathology, and high BMI.	Diagnostic interviews conducted annually over 8 years; risk factors assessed via self-report questionnaires at baseline

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					Predictive effects were medium-to-large	
O'Connor et al. (2016)	Ireland	290 Male 51.1% (n = 145) Female 48.9% (n = 139)	15-19 years; mean age 16.76 years (SD = 0.89)	AN, BN, BED	EDs were more stigmatized than depression and diabetes; BED was seen as a failure of self-control	Vignette-based scenarios referencing DSM conditions
Zanna et al. (2021)	Italy	346	10-15 years 15.0 (1.9) 14.8 (1.8) 10.6 (2.9)	AN-R, AN-A, ARFID	ARFID differed significantly in onset age, symptom profile, and comorbidity; shared vulnerability across groups	DSM-5 + standardized psychometrics (CDI, MASC, EDI-3, CBCL)
Ernst et al. (2017)	Germany	1,654	12-15 years (7th and 8th grade students)	AN, BN, EDNOS, OSFED	DSM-5 criteria resulted in higher prevalence; better differentiation between disordered and non-disordered groups	DSM-IV and DSM-5
Burt et al. (2020)	Australia	5,068 (FA: 402; OA: 4,586)	Adolescents (14-15 years)	OSFED-NES, AN, BN, others	EDs prevalent in both groups; OSFED-NES higher in First-Australians; associated with poor psychosocial quality of life	DSM-5

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Table 2. Risk of bias assessment using the Newcastle-Ottawa Scale

Studies	Selection (0-4)	Comparability (0-2)	Outcome/Exposure (0-3)	Total Score	Risk of Bias
Al Hourani et al. (2020)	3	1	2	6	Moderate
Andersen et al. (2023)	3	1	2	6	Moderate
Burt et al. (2020)	3	1	2	6	Moderate
Caspi et al. (2017)	2	2	3	7	Low
Couturier et al. (2023)	3	2	3	8	Low
Curzio et al. (2018)	2	1	2	5	Moderate
Diaz Muñoz (2021)	3	1	2	6	Moderate
Ernst et al. (2017)	3	1	2	6	Moderate
Lewis et al. (2019)	2	2	3	7	Low
Lie et al. (2021)	2	1	1	4	High
Murakami et al. (2016)	2	1	2	5	Moderate
O'Connor et al. (2016)	2	1	2	5	Moderate
Reinehr et al. (2019)	3	2	3	8	Low
Sandri et al. (2024)	3	1	2	6	Moderate
Vázquez-Velázquez et al. (2017)	2	2	3	7	Low
Yamamiya & Stice (2024)	3	2	3	8	Low
Yilmaz et al. (2019)	3	2	3	8	Low
Zanna et al. (2021)	2	1	2	5	Moderate

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Note. Studies using community or population-based samples (Lewis et al., 2019; Lie et al., 2021) were awarded 1 star for representativeness, whereas clinical samples (Caspi et al., 2017; Curzio et al., 2018) were not, due to potential selection bias. One star was awarded for control selection when controls were well defined and came from the same population (Diaz Muñoz, 2021). Use of standardized diagnostic criteria (DSM-5, EDDS) earned 1 star for exposure ascertainment, whereas self-reported or vignette-based diagnoses (Andersen et al., 2023; Murakami et al., 2016) did not. For cohort studies, an additional star was awarded when outcomes were not present at baseline (Brooke et al., 2021). Cross-sectional designs were exempt from this criterion. For comparability, studies adjusting for at least two confounders (age and gender) received 2 stars (Brooke et al., 2021; Diaz Muñoz, 2021), whereas those with limited adjustment (Reinehr et al., 2019) received fewer or no stars. For outcome/exposure, studies using clinical or independently assessed outcomes (e.g., health records and standardized scales) were rated higher (2-3 stars) (Diaz Muñoz, 2021, Curzio et al., 2018), whereas self-reported outcomes (Andersen et al., 2023) received 1 star. Cohort studies with sufficient follow-up and low attrition (Yamamiya & Stice, 2024) earned additional stars, whereas cross-sectional studies were not evaluated for follow-up.

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Nutritional Interventions to Address Deficiencies and Side Effects Associated with the Use of GLP-1 Receptor Agonist: A Systematic Review

Wareesha Khan[1*], Areej Tahir[1], Arooj Akram[1,] Nida Siddique[1], Kainat Bibi[1], Iqra Akhtar[1], Warda Khalid[1], Tara Khursheed[1]

[1] Department of Nutrition and Dietetics, National University of Medical Sciences, Rawalpindi

***Corresponding Author:** wareeshak11@gmail.com

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Abstract

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) have transformed the management of obesity and type 2 diabetes by promoting weight loss and improving cardiometabolic outcomes. However, gastrointestinal adverse effects and reduced dietary intake may contribute to micronutrient deficiencies, lean body mass loss, poor adherence, and compromised long-term health. This systematic review evaluated nutritional strategies for mitigating these risks in accordance with PRISMA guidelines. PubMed, Scopus, Web of Science, and Google Scholar were searched for studies published between 2010 and 2025. Eligible studies included adults receiving GLP-1 RAs and reporting nutritional deficiencies, gastrointestinal adverse events, dietary intake, lean-mass outcomes, or nutrition interventions. Data were extracted and synthesized thematically. Deficiencies of vitamin D, calcium, magnesium, potassium, iron, and vitamin B12 were frequently reported, together with nausea, vomiting, and delayed gastric emptying. Lean body mass represented approximately 30–40% of total weight loss, emphasizing the importance of adequate protein intake and structured resistance exercise. Individualized micronutrient supplementation, small frequent meals, dietary modification, and comprehensive nutrition counseling helped correct deficiencies, manage symptoms, and improve treatment adherence. Evidence and implementation gaps were particularly apparent for socioeconomically disadvantaged individuals, older adults, and pediatric populations. Although GLP-1 RAs provide substantial metabolic benefits, they may create clinically important nutritional risks. Routine nutritional assessment, symptom monitoring, protein optimization, appropriate supplementation, and exercise support are therefore essential. Future research should establish standardized protocols and evaluate integrated nutrition-care models across diverse real-world populations.

Keywords: GLP-1 receptor agonist, Nutritional deficiencies, Protein intake, Micronutrient supplementation, Gastrointestinal side effects, Obesity, Type II diabetes.

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1. Introduction

The agonists of glucagon-like peptide-1 receptor (GLP-1 RAs) have emerged as a central component of the treatment plan of obesity and type 2 diabetes, with semaglutide, liraglutide, and tirzepatide showing significant effectiveness in the elicitation of clinically meaningful weight loss and cardiometabolic index improvement (Gorgojo-Martinez et al., 2022). Weight losses of between 5 -18 per cent placebo-adjusted have been reported, which coincides with improvements in glycaemic control, lipid metabolism and renal status. Due to their clinical importance, GLP -1 RA have been added to the Essential Medicines List of the World Health Organization, supporting their implementation as part of a multidisciplinary, long-term obesity management strategy (World Health Organization, 2025).

Despite these benefits, GLP-1 RA treatment is associated with severe nutritional and gastrointestinal safety incidents. Appetite suppression, early satiety, gastrointestinal issues such as nausea, vomiting, diarrhoea and constipation do not spare 40-70% of patients and trigger reduced caloric intake, micronutrient loss, and discontinuation of the therapy (Gorgojo-Martinez et al., 2022; Ismaiel et al., 2025). There is evidence that there will be a considerable number of patients who develop a deficiency in vitamin D, calcium, magnesium, iron, and vitamin B12 during the first year of treatment, and disproportionate depletion of lean body mass further worsens metabolic health and physical functionality (Butsch et al., 2025; Spreckley et al., 2025).

The pharmacodynamic effectiveness of GLP-1 RAs is undeniable, however there is a lack of a rigorous dietary curriculum. Instead of being proactive in using evidence-based treatments, modern healthcare practice has been more reactive in addressing deficiencies and GIT sequelae (Mozaffarian et al., 2025). Therefore, there is a pressing need of specific nutritional measures, which could be optimized protein intake, micronutrient supplement, and changes in the diet directed at improving gastrointestinal tolerability. The current study critically examines these interventions in a bid to prevent GLP 1 RA related failures and adverse outcomes thus promoting safe, efficient and effective treatment.

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2. Materials and Methods

2.1 Study Design and Review Framework

To provide transparency, methodological rigor, and reproducibility, the systematic literature review was conducted in compliance with the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The PRISMA protocol provides a systematic and universal way of determining relevant literature, filtering studies, using inclusion and exclusion criteria, and retrieving data as well as synthesizing outcomes. A rigorous compliance with the given framework can increase the reliability and validity of the review process by reducing the selection bias, recording the decision pathways, and providing the methodological reliability (Thomé et al., 2016).

A systematic review approach was chosen due to the emerging nature of the studies that examine nutritional interventions in patients receiving GLP-1 receptor agonists, in order to gather fragmented evidence and come up with a synthesis of dietary interventions aimed to alleviate the effects of micronutrient deficits, gastrointestinal adverse effects, and lean mass loss in patients treated with GLP-1 RA. The thematic framework was used to analyze and identify, interpret, and organize recurrent patterns in different study designs.

2.2 Data Sources and Search Strategy

The literature review included academic databases as PubMed, Scopus, Web of Science, and Google Scholar that are known to be covered with a significant amount of clinical, nutritional, and pharmacological inquiries (Gusenbauer and Haddaway, 2020). The search strategy focused on articles published in 2010-2025 to include current evidence related to GLP -1 RA therapy and nutritional management. Combination of search terms was transmitted by means of keywords, Boolean operators, and MeSH terms. Keywords like, GLP-1 receptor agonists, semaglutide, liraglutide, tirzepatide, nutritional deficiency, micronutrient deficiency, lean mass loss, sarcopenia, gastrointestinal side effects, nutrition intervention, protein assimilation, micronutrient supplementation, dietary modification, dietary strategies, obesity pharmacotherapy, and nutritional management. Each database had its search strings modified in order to achieve maximum sensitivity and relevancy. Additional articles were manually screened as reference lists of the qualifying articles and pivotal reviews to provide completeness.

2.3 Eligibility Criteria

The selection of the studies was based on PECO model (Population, Exposure, Comparator, Outcome), which offers a systematic way of finding relevant studies.

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Table 1: Eligibility Criteria Based on PECO Framework

Criteria	Inclusion	Exclusion
Population	Patients using GLP-1 RAs for obesity or type 2 diabetes	Studies involving animals, or non-GLP-1 RA therapies
Exposure	Use of GLP-1 receptor agonists (e.g., semaglutide, liraglutide, tirzepatide) associated with nutritional deficiencies or side effects	Studies examining GLP-1 physiology unrelated to medication use
Comparator	With or without a comparator group	—
Outcomes	Nutritional deficiencies, GI adverse events, protein intake, micronutrient status, lean mass outcomes, or effectiveness of nutritional interventions	Studies without nutrition-related outcomes
Study Design	Randomized controlled trials, observational studies, clinical guidelines, qualitative studies, and systematic reviews	Non-peer-reviewed articles, conference abstracts, editorials, commentaries

2.4 Study Selection and PRISMA Flow Process

The results of all searches were imported into endnote software and duplication removed. Relevance of titles and abstracts was done and full-text evaluated against the eligibility criteria. The causes of exclusion were recorded at every phase. A PRISMA flow diagram was used to record the selection process summarizing the number of identified, screened, eligible and included studies in the final synthesis.

2.5 Quality Assessment

The quality of the incorporated studies was evaluated by the modified version of Quality Assessment Tool of Studies of Varying Design (QATSDD). The tool was chosen due to its suitability for incorporating different study designs within a review. Clarity of purpose, technique suitability, sample description, data collection procedure, analytic rigor, and findings relevance were among the evaluation criteria. Each study's quality score was determined, but it wasn't the sole criterion for inclusion in the synthesis.

2.6 Data Extraction Process and Thematic Analysis

It was created as a structured data-extraction tool to offer the consistency and rigor of approach. The tool collected information on study characteristics, demogphics, the type and dosage of GLP-1 receptor agonist (GLP-1 RA), reported nutritional deficiencies, nutritional intake, gastrointestinal side effects, and specific nutritional interventions under investigation (e.g., protein optimization, micronutrient supplementation, dietary counseling). Error was reduced by cross-verifying the extracted data. Because the study design, study outcomes, and intervention modalities varied, a narrative synthesis was conducted and backed by an official theme analysis. This analytical approach made it possible to identify successful nutrition interventions for GLP-1 RA patients and helped with the methodical interpretation of the results of various techniques.

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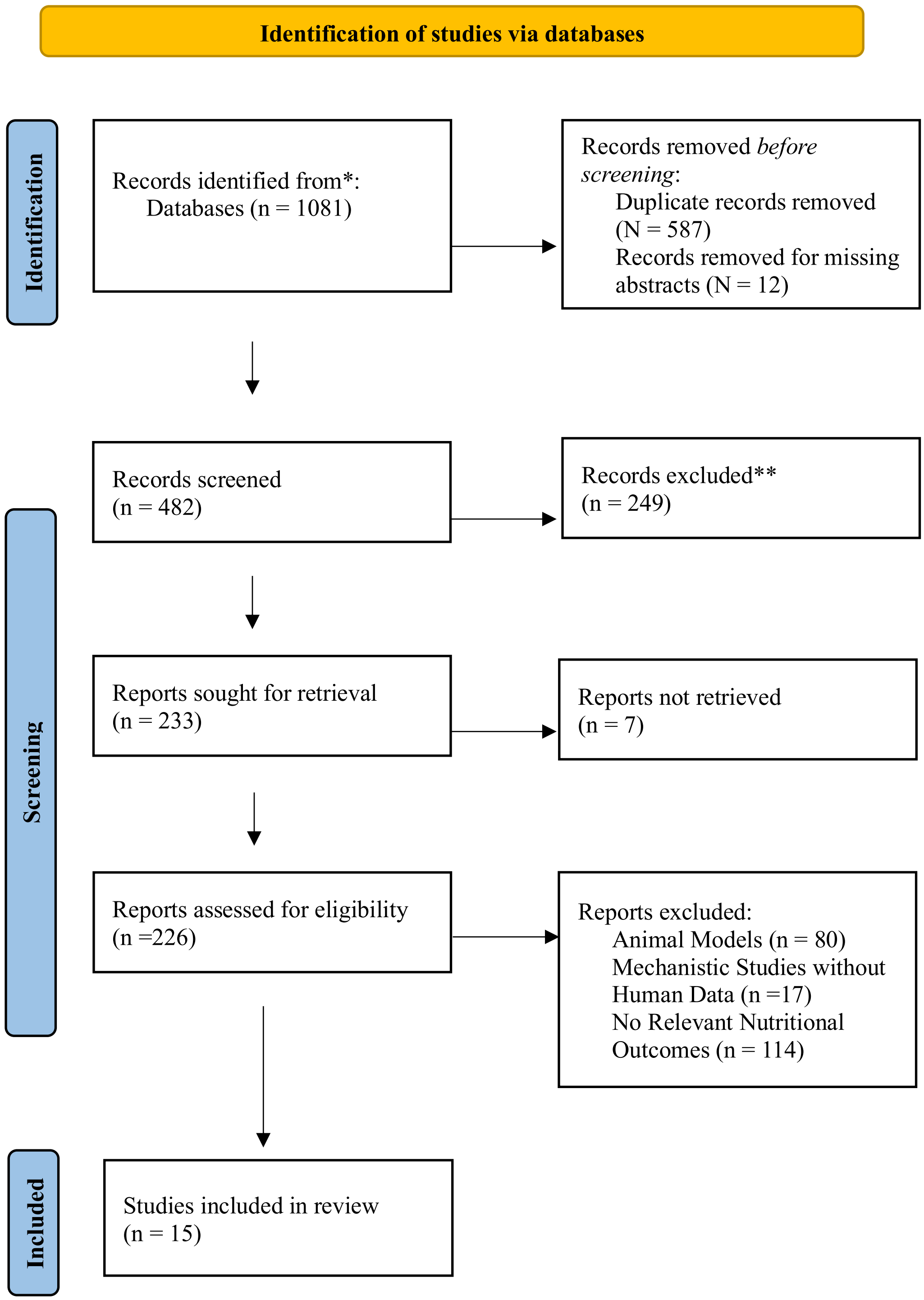

Figure 1: PRISMA Flow Diagram

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3. Results

Significant trends in the dietary effects, physiological ramifications, suggested interventions, and population-specific issues of GLP-1 RA therapy were identified by this synthesis of the evidence in the evaluated papers. The prevalence and types of dietary deficiencies, gastrointestinal and metabolic side effects, nutritional and lifestyle therapies, and special populations and implementation gaps were the four primary topics that emerged.

3.1 Summary of Themes

3.1.1 Prevalence and Types of Nutritional Deficiencies

In all the works that were present in the review, nutritional deficiencies were continuously mentioned to be a severe effect of GLP-1 RA treatment which is mostly due to the decrease in the caloric intake, suppression of the appetite, and change in the dietary habits. Butsch et al. (2025) presented strong real-world data which stated that 12.7% of patients had at least one deficiency in six months, which rose to 22.4% in the first year of treatment. Vitamin D deficiency became the most common with incidence rates of 7.5 percent at six months and 13.6 percent at 12 months and inadequate intakes of calcium, iron, choline, magnesium, fiber, and potassium were reported by Johnson et al. (2025). These micronutrient deficiencies indicate physiological alterations caused by GLP 1 RAs as well as dietary imbalance. Kennedy et al. (2025) also focused on the danger of micronutrient deficiency, whose fast satiety can negatively affect the already inappropriate nutrient baselines. Mozaffarian et al. (2025) have noted that caloric limitation and lack of food variety in the course of treatment may undermine muscle and bone health, which is why the monitoring of micronutrients is essential. Taken together, the evidence suggests that although GLP-1 RA treatment is effective in the management of weight, it is linked to a significant risk of nutrient deficiency that should be assessed proactively and planned in the diet.

3.1.2 Gastrointestinal and Metabolic Side Effects

Gastrointestinal disturbances are one of the most consistent adverse effects of the studies reviewed. Mozaffarian et al. (2025) and Xie et al. (2025) noted that Nausea, vomiting, slow gastric emptying,

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constipation and abdominal discomfort were common particularly when starting and increasing the dose. Additionally, gastrointestinal side effects are the significant predictor of treatment discontinuation and poor adherence. Furthermore, study by Xie et al. (2025) also showed that the use of GLP 1 RA was linked to higher risks of gastrointestinal disorders, pancreatitis, nephrolithiasis, hypotension, syncope, and arthritic symptoms compared to usual antihyperglycaemic treatments. However, such risks were accompanied by a wide range of metabolic advantages, including a lower number of cardiovascular events, a better lipid profile, and a decreased risk of neurocognitive disorders. Mechanistic data provided by Moiz et al. (2025) and Ilias et al. (2025) made it clear that much of this side effects is an expansion of the physiological activity of GLP-1, especially delayed emptying of the stomach, control of central appetite, and hormonal control. Reduced gastrointestinal pace, though advantageous to glycaemic management and satiety, is a direct cause of nausea and early satiety, and decreased food consumption, which further increases nutritional risk. Schooling et al. (2025) and Wang and Wang (2025) emphasized the importance of AMPK (AMP-activated protein kinase) activation and metabolic remodeling, which might be the cause of other system-wide effects other than the gastrointestinal system.

3.1.3 Nutritional and Lifestyle Interventions

The need to use organized nutritional and lifestyle interventions to reduce nutritional risks as well as maximize therapeutic outcomes was stressed in all reviewed studies. Evidence-based nutritional interventions that have been suggested by Kennedy et al. (2025) include protein prioritization, gradual fiber intake, small frequent meals, and monitoring of micronutrients, which are deemed essential to maintain lean body mass and reduce gastrointestinal discomfort. Mozaffarian et al. (2025) also emphasized baseline measurements, such as the quality of the diet, emotional eating habits, muscle strength, and social determinants of health, and continuous lifestyle observation during the therapy. It was found that interdisciplinary care models were critical to effective management. However, Butsch et al. (2025) found that patients with dietitian visits had a higher likelihood of reported deficiencies, which probably was a consequence of the more frequent screening instead of the worsening of the nutritional condition. Recommended supportive

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interventions were resistance training to maintain muscle mass, structured exercise, telehealth-based dietary counseling, and Food-is-Medicine programs. Another issue mentioned in the literature is that more intervention strategies need to be developed, specific to the emerging co-agonists (e.g., GLP-1/GIP dual agents) that can be more effective in terms of appetite suppression. It is proposed by mechanistic studies (Moiz et al., 2025; Liu, 2024) that nutrition-oriented behavioural interventions will be even more important as future pharmaceuticals will be more potent and prevent severe deficits and uphold metabolic health.

3.1.4 Special Populations and Implementation Gaps

Some studies revealed substantive implementational gaps, especially among users of pediatric medicine, those with limited nutritional literacy and those with structural constraints. Kennedy et al. (2025) highlighted a significant evidence gap in children and adolescent diets, which is despite the increasing prescription of GLP-1 RA in these populations. The increased susceptibility of pediatric patients is attributed to their continuous development, increased nutrient needs, and increased vulnerability to deficiencies caused by appetite. Mozaffarian et al. (2025) have found structural barriers that affect treatment outcomes to be broader, such as food insecurity, inadequate access to dietitians, low culinary skills, and inequity in drug affordability. These problems impact low-income populations to a disproportionate extent and could contribute to the increase in inequalities in managing obesity and metabolic diseases. Furthermore, West et al., (2025) and Xie et al., (2025) reported that neurological mechanisms of GLP-1 RAs provided gaps in understanding the long-term effects on cognitive functions, particularly in the vulnerable population. Meanwhile, Chen et al. (2025), Zheng et al. (2024) and Ussher and Drucker (2023) have indicated the growing therapeutic indications in renal, cardiovascular, hepatic, neurological, and inflammatory conditions and it is not clear how nutritional management needs to be tailored to various comorbidities.

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4. Discussion

The results of the review provide great insight into the nutritional risks associated with the use of GLP-1 receptor agonist (GLP-1RA) treatment, explaining the main thematic issues: the nutritional deficiencies, gastrointestinal and metabolic adverse effects, changes in dietary intake, nutritional risks related to weight, and the efficacy of nutritional interventions. Taken together, these themes highlight the sophisticated physiological and behavioural processes through which GLP -1 RAs regulate nutrient status.

4.1 Nutritional Deficiencies and Micronutrient Risks

The most significant result of the review is that deficiencies in vitamin D, calcium, magnesium, potassium, iron, and vitamin B12 are high among users of GLP-1 RA. These are observations that are consistent with the new research. A number of studies reported micronutrient imbalances in patients undergoing GLP-1RA treatment, especially those treated with semaglutide or liraglutide to manage obesity and diabetes. Indicatively, Lupianez-Merly et al. (2024) found higher incidences of vitamin D and magnesium deficiency during treatment, which is similar to our outcome of up to 22 per cent of users developing at least one deficiency in the first year of treatment. Similarly, it was emphasized by Gentinetta et al. (2024) that delayed gastric emptying is one of the major actions of GLP-1 agonists, which may decrease nutrient absorption, especially fat-soluble vitamins like vitamin D.

The observation of the review that the most prevalent is vitamin D deficiency is also correlated with the overall epidemiological data that show high baseline vitamin D insufficiency among obese and type 2 diabetes patients. However, other reports indicate that GLP-1 treatment can indirectly increase the levels of vitamin D through weight loss and reduced fat-soluble vitamin sequestration in adipose tissues (Ali et al., 2024). This is contrary to our findings that suggest that the extent of appetite inhibition and dietary restraint upon GLP -1RAs might counter any weight-loss-related changes in vitamin D bioavailability. The contradicting evidence thus supports the necessity of

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additional controlled experiments that isolate the metabolic versus dietary behaviour-mediated actions of GLP -1 RAs.

The review was also characterized by iron and B12 deficiencies. These results are in agreement with Bain et al. (2023), who have found that levels of ferritin and B12 decrease in GLP-1 RA users, especially those with gastrointestinal side effects like nausea, vomiting, or lack of animal-source food intake. In contrast, Chapman et al. (2016) did not observe clinically significant reduction in B12 and indicated that the risk of deficiency could be determined by the dietary patterns at baseline or co-administration of metformin, which has an independent adverse effect on B12 absorption. This difference suggests that the decrease in B12 may not necessarily be due to GLP-1 physiology but may be increased by dietary modifications with treatment.

4.2 Gastrointestinal and Metabolic Side Effects

The results of this review show that gastrointestinal (GI) disturbances represent the most frequently reported adverse effects in GLP -1 -RA users, which is well-supported across the literature. Similarly, Sun et al., (2015) and Gartner et al., (2025) also point to the same adverse events (nausea, vomiting, constipation, and abdominal discomfort) as typical adverse events, especially in the initial stages of treatment. These symptoms have been widely mentioned as the most common reason leading to discontinuation of treatment, which emphasizes their clinical importance. Chun and Butts (2020) support this point and emphasize that dose-based GI intolerance is one of the primary obstacles to persistent adherence.

Furthermore, Filippatos et al. (2015) disclose greater risks of pancreatitis, nephrolithiasis, hypotension, and syncope in GLP-1RA users, but such incidences are still relatively low. However, they should be understood in the framework of the overall therapeutic process. Ma et al. (2021) and Iorga et al. (2020) also reporting significant metabolic positive outcomes, such as cardiovascular mortality, improved lipids, and possible neuroprotection. This duality is indicative of a crucial clinical trade-off, in that, there are side effects, but they are accompanied by substantial metabolic benefits.

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These side effects are further put in perspective by mechanistic insights. Kim and Yoo (2025) and Zhao et al. (2021) show that a substantial number of GI symptoms are the direct results of the pharmacological effects of GLP-1. Slow gastric emptying, central appetite inhibition, and vagal signaling changes are concomitant causes of nausea, early satiety, and dietary intake. Such mechanisms can also increase micronutrient deficiencies, which connect metabolic activities with nutritional risk. Additionally, Kim and Yoo (2025) and Fadel et al. (2025) point out that, there is the implication of AMPK activation and metabolic remodelling, which explains the systemic manifestations of fatigue, hypotension, or musculoskeletal pain. The literature justifies the idea that GLP-1 RAs have predictable GI and metabolic side effects based on their physiological activity, and the importance of close dose adjustment and nutritional follow-up.

4.3 Altered Dietary Intake and Appetite Suppression

The decreased total dietary intake and intake of foods rich in micronutrients is in line with the established anorexic action of GLP-1 RAs. Some of the studies such as Camilleri (2024) and Horowitz et al. (2012) have shown that energy intake reduces significantly after GLP-1 therapy, mainly due to early satiety, slow gastric emptying, and reduced hedonic eating. Our results expand this evidence by demonstrating that the decreasing consumption of foods high in calcium, magnesium, iron, and vitamin B12, including dairy products, leafy vegetables, legumes, and meat, is not proportional. This is in line with behavioural outcomes of Bettadapura et al. (2025) who found that patients undergoing GLP-1 therapy often develop aversions to meat and high-density foods, which might be the reason why B12 and iron deficiencies are more prevalent.

The decrease in the consumption of micronutrient-contained foods also aligns with the research that demonstrates that GLP-1 treatment changes the dietary habits to simple, easy to digest foods which tend to be deficient in essential minerals (Christensen et al., 2024). Nevertheless, other studies state that decreased consumption is a therapeutic outcome of reduced intake desired as a part of weight loss and enhanced metabolic well-being (Ard et al., 2021; Wong et al., 2025). Although this view is correct in terms of weight-management perspective, the results of this review have indicated that the nutritional implication deserves more clinical consideration.

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4.4 Weight-Loss-Related Nutritional Risks

The weight loss was long related to changes in micronutrient metabolism, such as the depletion of vitamin D reserves due to the decrease in the volume of adipose tissue, the decline in the levels of iron caused by the decrease in consumption, and the disruption of the electrolyte balance (Ciobarca et al., 2024). The current evidence suggests that patients receiving GLP-1 receptor agonists (GLP-1 RAs) seem especially vulnerable to the deficiencies, which is in line with the previous studies that revealed that the rate and the extent of weight loss correlate with the occurrence of micronutrient deficiency (Febrey et al., 2025).

The current body of literature is however divided on whether there is clinical importance on the nutritional risks induced by weight-loss. Some of the research assumes that weight loss induced by GLP-1 is more metabolically favourable and slower than surgery or extreme diets (de Almeida et al., 2024), which may help to avoid dangerous deficiencies. On the other hand, Klair et al., (2023) indicate that semaglutide may provide weight loss just as effective as bariatric surgery in specific patients, thus increasing nutritional risk more than previously predicted (2023). The findings agree with Moetaz et al. (2025), which suggest that clinicians should use bariatric-style-nutritional-monitoring protocols in the management of GLP-1-induced weight loss.

4.5 Efficacy of Nutritional Interventions

The efficiency of the supplementation and lifestyle intervention demonstrates the gaps in the contemporary clinical practice. This has been proven to be effective, with regular monitoring of vitamin D supplementation being found to fix deficiency on GLP-1 RA users (Chavez et al., 2025), which is why our results support the idea that targeted supplementation can make the difference. Conversely, dietary counselling has shown to be less effective with generalized use of multivitamins without behavioural modification, hence the significance of behavioural modification towards the achievement of adequate nutrient status (Acero et al., 2025).

Dietary counselling has been found to be specifically effective, in line with Ismaiel et al. (2025), who report the need to monitor patient with a rapid weight loss, either surgically or pharmacologically induced, under the guidance of a dietitian. The results of the review also support

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the position of Chavez et al. (2025), who propose to include nutritional assessment in the GLP-1 RA treatment protocols, as the negative effects associated with nutrients are increasingly being recognized.

However, some studies, including those by Brown et al. (2024) and Karakasis et al. (2025), claim that routine supplementation might not be necessary in case of no clinically significant deficiencies. Such studies are usually earlier than the common use of GLP-1 agonists in higher doses such as semaglutide and tirzepatide. The present review thus confirms the growing view that nutritional surveillance should become a norm due to the increasing doses and the extended use of GLP-1 drugs.

5. Conclusion

GLP-1 receptor agonists are a disruptive therapeutic agent in obesity and type 2 diabetes, providing significant weight loss and metabolic effects. However, according to this review, their anorexia-inducing and gastrointestinal action presents significant nutritional difficulties. It has been proven that micronutrient deficiencies, specifically vitamin D, calcium, magnesium, potassium, iron, and vitamin B12 are highly prevalent, which is mainly caused by the decreased consumption of these nutrients in the diet, the change in food preferences, and the weight loss. These deficiencies are enhanced by gastrointestinal adverse events such as nausea, vomiting, constipation and early satiety as some of the contributing factors to treatment discontinuation. Moreover, the excessive loss of lean body mass in the case of GLP-1 RA treatment is a matter of concern in terms of sarcopenia, the decline in metabolic rate, and physical functioning, in which case it is essential to maintain sufficient protein consumption and resistance-based exercise.

The review establishes that the lack of micronutrients, protein optimisation, small frequent meals, and dietitian-led counselling, as structured nutritional interventions, have the potential to alleviate deficiencies and aid adherence. The baseline assessment of dietary intake, constant monitoring, and individual approaches play a key role in the retention of lean mass, the avoidance of micronutrient depletion, and metabolic health. However, there are still gaps in evidence, especially in the cases of pediatric patients, socioeconomically disadvantaged, and those taking newer dual

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agonists, which imply that the specific dietary recommendations should be provided, and access to nutritional support should be equal.

In addition, proactive and evidence-based nutritional care should be included in the GLP-1 RA therapy. Individualized supplementation, systematic dietary evaluation, and lifestyle modifications are critical in order to make sure that the deep cardiometabolic effects of GLP-1 RAs are not missed by avoidable dietary complications. Such comprehensive strategies will improve treatment safety, adherence, and long-term outcomes to become a template of optimised, sustainable obesity and diabetes management.

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Author Contributions

W.K did the conceptualisation, data extraction, analysis and manuscript drafting; A.T, A.A, N.S, K.B, I.A and W.K extracted and reviewed the data and did manuscript revision. T.K supervised, revised and approved the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interest

The authors declare no potential conflicts of interest.

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Ethical Approval

Not applicable

Informed Consent

Not applicable.

Data Availability Statement

All data are derived from published studies cited in the references. No new data were generated.

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Cold-Chain Logistics Optimization for Fresh Horticultural Produce: Evidence, Models, Trade-offs, and Implementation Priorities

Faizan Tanvir*[1], Asia Laitf Mughal[1], Rimsha Shabbir [1]

[1] Institute of Food and Nutritional Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

***Corresponding author:** faizantanvir344@gmail.com

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Abstract

Cold-chain failures accelerate water loss, respiration, senescence, physiological injury, and microbial spoilage in fresh fruits and vegetables, but the reported outcome depends on the endpoint and supply chain boundary. This structured narrative review asks how physical controls, digital tools, and optimization models should be selected when biological fit, measured loss and quality, food safety, cost, energy, infrastructure, and evidence readiness are considered together. Searches of scholarly and institutional sources were updated to 22 July 2026. Primary experiments, field deployments, optimization studies, reviews, and authoritative reports were classified by design, validation scale, and endpoint; a 32 record analytic inventory is supplied separately. Evidence indicates that commodity appropriate removal of field heat, stable storage and transport conditions, airflow management, suitable relative humidity, hygienic handling, and compatible packaging remain the control foundation. Sensors, radio frequency identification, block chain, and digital twins provide different information functions; none directly controls temperature or establishes microbiological safety. Optimization models can clarify trade-offs among cost, delivery time, deterioration, service, and emissions, but most evidence remains simulation or case based and rarely verifies realized food loss in commercial operation. The proposed readiness matrix links loss mechanisms and interventions to data needs, decision ownership, resource context, and accountable outcomes. Priorities are field trials, product temperature monitoring, explicit uncertainty and quality decay models, validated escalation procedures, transparent lifecycle and financial boundaries, and baselines reported with units. Investment should progress from reliable biological and operational control to monitoring, prediction, and optimization, with adoption contingent on measured improvement over a defined comparator.

Keywords: fresh produce; postharvest loss; refrigeration; precooling; monitoring; supply chain optimization; evidence readiness

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1. Introduction

Reducing loss in fruit and vegetable supply chains is simultaneously a food security, nutrition, income and environmental objective. The concepts must nevertheless be kept distinct. The Food and Agriculture Organization (FAO) uses *food loss* for reductions in food quantity or quality from production up to, but not including, retail, whereas *food waste* is associated mainly with retail, food service and consumers (FAO, 2019). The FAO indicator for 2023 estimates that 13.3% of food is lost globally after harvest and before retail, but that aggregate is not a universal rate for every commodity, country or supply chain stage (FAO, 2025). The United Nations Environment Programme (UNEP) separately estimated 1.05 billion tons of consumer level food waste in 2022 (UNEP, 2024). Combining these denominators or transferring either estimate to a particular horticultural chain would be misleading.

Fresh horticultural produce warrants a dedicated synthesis because it remains biologically active after harvest. Respiration consumes substrates, transpiration removes water, ethylene can accelerate ripening or senescence, mechanical injury increases susceptibility to decay, and microorganisms exploit damaged or wet tissue. Temperature affects most of these processes, but "colder" is not always "better": chilling-sensitive tropical and subtropical commodities may develop physiological injury above their freezing point, while excessive humidity, condensation, poor sanitation or incompatible mixed loads can create other quality and safety problems (Gross et al., 2016; Onwude et al., 2020). Meat, fish and dairy have materially different deterioration pathways and hazard controls; they are therefore outside this review.

The cold chain is a sequence of linked operations rather than a single refrigerator. Harvest time and maturity, shade and field delay, precooling, grading, packaging, cold storage, loading, refrigerated transport, cross-docking and retail rotation influence the temperature history and remaining commercial life of a load. A strong downstream unit cannot fully reverse delayed precooling, freezing injury, bruising or contamination introduced upstream. Conversely, a technically efficient cold room can be uneconomic or environmentally counterproductive if it is under-utilized, poorly maintained, charged with a high-impact refrigerant, supplied by an unreliable carbon-intensive grid, or installed without a route to market (FAO and UNEP, 2022).

Recent reviews describe advances in sensing, imaging, packaging, digital twins and cold-chain management (Onwude et al., 2020; Han et al., 2021; Mustafa et al., 2024), while the optimization literature has expanded rapidly across facility location, inventory, production, routing and energy decisions (Iyer and Robb, 2025). However, three interpretive errors remain common. First, a sensor that detects an excursion is treated as if it prevented loss. Second, an objective-function improvement in a simulation is treated as realized food saved. Third, records secured by blockchain are treated as inherently accurate. Each technology occupies a different part of the causal chain and depends on calibration, data coverage, decision rules, personnel and feasible corrective action.

This review narrows the topic to whole and minimally processed fresh fruits and vegetables from harvest through distribution and retail entry. It covers established physical interventions (precooling, storage, transport and modified-atmosphere packaging), information tools (sensors, Internet of Things [IoT], identification, traceability, prediction and digital twins), logistics

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optimization, sustainability, economics and end-of-life routing. The principal outcome is not a single pooled effect; it is a decision framework that asks whether an intervention is biologically appropriate, operationally feasible, supported by evidence at the claimed scale, and linked to an accountable endpoint.

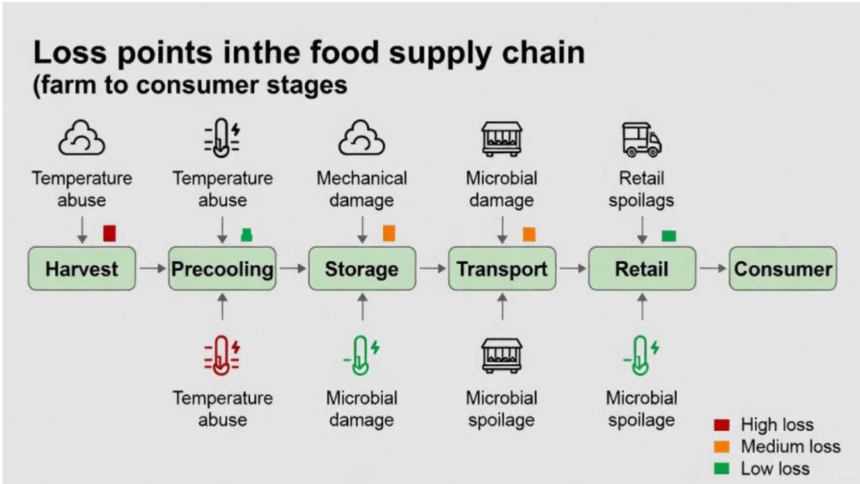

Figure 1: Loss points in the food supply chain (Farm to consumer stage)

The research question is: How should cold-chain interventions and optimization models be selected for fresh horticultural produce when biological fit, food safety, measured physical and quality loss, cost, energy, infrastructure and evidence readiness are considered together? The novelty is an evidence- and readiness-based synthesis that connects the loss mechanism and commodity to the control action, data architecture, model, trade-offs, validation scale and adoption gate.

2. Review approach and evidence classification

2.1 Search scope and source selection

This review was conducted as a structured narrative review rather than a systematic review or meta-analysis. Targeted searches were updated to 22 July 2026 using PubMed, Google Scholar, ScienceDirect, publisher platforms and institutional repositories. Search concepts were combined in groups: ("fresh produce" OR fruit OR vegetable OR horticultural) AND ("cold chain" OR precooling OR refrigerated transport OR cold storage OR "modified atmosphere") AND (loss OR quality OR shelf life OR safety); and ("cold chain" OR perishable) AND (optimization OR routing OR network OR inventory OR "quality decay" OR "digital twin" OR IoT OR sensor OR blockchain). Backward and forward citation checks were used for foundational studies and corrected records identified during the claim-to-source audit.

English-language peer-reviewed articles, authoritative reports and technical handbooks were eligible. The principal date window was 2014-22 July 2026, with earlier sources retained when they defined a method, postharvest requirement or optimization formulation still used in the recent literature. Sources were included when they specified a relevant commodity or fresh produce

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context, cold-chain stage, intervention or model, and at least one interpretable outcome or decision variable. Sources were excluded when they concerned only meat, fish, dairy or pharmaceuticals; ambient processing unrelated to a cold chain; consumer waste without a postharvest logistics link; or asserted a performance benefit without enough methodological information to interpret the claim.

Because the review was developed iteratively and was not based on a prospective protocol or a single exported bibliographic database, candidate identification and exclusion counts were not retained. Inventing a PRISMA-style flow would be inappropriate. Instead, the final analytic inventory is made auditable: the supplementary table lists 32 core records by source type, commodity/context, scale, intervention or model, comparator, measured endpoint and limitation. Authoritative sources used only to define food loss/waste or commodity storage guidance are identified separately in the reference list. This transparent limitation means the review should be interpreted as a critical, decision-oriented synthesis, not an exhaustive prevalence estimate.

2.2 Evidence categories and readiness

Evidence was classified before claims were synthesized, which includes institutional indicators, handbooks and standards that define boundaries or recommended conditions. Review which includes narrative, scoping or systematic synthesis; useful for breadth but not direct evidence that an intervention caused an outcome. Laboratory or pilot experiment for controlled product or package evaluation, usually with defined temperatures and quality measurements but limited logistics complexity. Commercial field deployment or observational study for technology operated in a working chain; may demonstrate feasibility and exposure detection but needs a comparator to attribute loss reduction. Optimization model or simulation having mathematical formulation evaluated with synthetic, benchmark or case data; supports prospective decisions but does not alone demonstrate food saved. Conceptual framework, interview or implementation case identifies mechanisms, opportunities and constraints; causal performance claims require separate outcome evidence.

Four readiness levels are used in the synthesis: R1, conceptual (function and proposed mechanism); R2, laboratory or simulation validated; R3, pilot or case validated in a relevant operational setting; and R4, replicated commercial outcome evidence with a defined comparator and implementation conditions. Readiness is assigned to the specific claimed use, not to the technology name. For example, temperature sensing is mature for measurement, but a sensor driven shelf life decision may be only R2 or R3 if the quality model and escalation protocol have not been validated for the commodity and route.

2.3 Outcome definitions and causal language

The review uses the following endpoints which includes Physical loss, mass or units removed from the food supply chain, reported against a stated denominator. Quality loss, change in market or sensory attributes such as firmness, color, flavor, turgor or visible defects; market rejection is reported separately because grading rules vary. Nutritional deterioration, measured change in nutrients or bioactive compounds, not inferred from appearance alone.

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Safety non-compliance includes failure of a validated hazard-specific microbiological, chemical or physical criterion. Temperature history can inform risk but does not prove safety. Shelf life is the time to a prespecified quality or safety limit under stated conditions; an extension is meaningful only relative to a comparator and endpoint. Economic loss is reduction in saleable yield, revenue or margin, or additional cost; price and quantity components should be separated.

Terms such as demonstrated, reduced or improved are reserved for studies that measured the relevant outcome against a comparator. Detected, predicted, optimized and proposed describe monitoring, modeling and conceptual findings without implying realized loss prevention.

3. Biological and measurement basis for cold-chain decisions

3.1 Commodity, maturity and initial condition

Cold-chain design begins with the product, not the device. Cultivar, maturity at harvest, preharvest weather, field temperature, size, surface-to-volume ratio, respiration rate, ethylene sensitivity and mechanical damage affect cooling and deterioration. A route that is suitable for mature green tomatoes is not automatically suitable for berries, leafy vegetables or chilling-sensitive mangoes. Target temperature and relative humidity should therefore be drawn from commodity-specific guidance and validated against the intended market duration (Gross et al., 2016).

Initial load temperature is particularly important. The refrigeration demand required to remove field heat can exceed the heat entering later through insulation, doors and respiration. Delayed precooling can consume a substantial portion of remaining commercial life, whereas loading warm produce into a vehicle intended only to maintain temperature may create slow and spatially uneven pull down. Reporting only the thermostat set-point conceals this difference.

3.2 Heat, moisture, gases and microbial hazards

The time–temperature history affects respiration, senescence and growth of spoilage organisms, but air and product temperatures are not interchangeable. Air may respond quickly to compressor cycles and door openings while the product core changes slowly. Sensor position near an evaporator, ceiling or door can therefore misrepresent the warmest or slowest cooling part of a pallet. Studies and audits should report sensor accuracy, calibration, location, sampling interval, missing data and whether product, pulp, surface or air temperature was measured (Mercier et al., 2017; Badia-Melis et al., 2018).

Relative humidity is similarly double-edged. High humidity limits water loss and wilting, but surface condensation can promote decay, weaken cartons and transfer microorganisms. Airflow must remove heat without creating excessive dehydration; package vents and pallet stacking must match the cooling method. Respiration also produces heat and carbon dioxide, and mixed loads may expose ethylene sensitive commodities to ethylene-producing fruit. The appropriate control is consequently a combination of temperature, humidity, airflow, gas exchange, hygiene and compatibility.

Food safety and quality must remain separate. Cooling generally slows microbial growth but does not sanitize contaminated produce. Hydrocooling and flume water can spread contamination if

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disinfectant concentration, organic load, temperature and water replacement are not controlled. Modified atmospheres can slow respiration, yet excessively low oxygen or high carbon dioxide can cause anaerobic metabolism, off-flavors or tissue injury. No temperature excursion algorithm should be used to release a load as "safe" without a validated hazard model and the relevant process controls.

4. Physical interventions: the control foundation

4.1 Precooling and first-mile handling

Precooling removes field heat soon after harvest using room cooling, forced-air cooling, hydrocooling, vacuum cooling, package icing or combinations. Selection depends on commodity tolerance to water and vacuum, package ventilation, throughput, water quality, energy and capital. Forced-air systems can improve cooling uniformity when vents align and air bypass is controlled; hydrocooling is rapid for water tolerant produce but requires hygienic water management; vacuum cooling is effective for high surface area leafy vegetables but has product and equipment constraints (Duan et al., 2020).

Cooling performance should be expressed as product temperature pull down for example, time to seven-eighths cooling rather than only room set point. Minimum reporting includes cultivar and maturity, harvest and load temperature, target temperature, cooling medium, air or water flow, package and stacking, mass, cooling time, energy use and quality outcome after the intended distribution period. Without these data, a "precooled" treatment cannot be reproduced or compared.

Low-resource chains may benefit more from shade, shorter field delay, ventilated crates, coordinated pickup and a correctly sized shared precooler than from an advanced analytics platform. The decision criterion is avoided deterioration per unit of total cost and energy under realistic throughput, not technological novelty.

4.2 Storage and transport

Cold stores and vehicles must maintain a commodity appropriate envelope while allowing heat removal and air circulation. Important design and operating variables include insulation, refrigeration capacity at local ambient conditions, evaporator and fan configuration, defrost, door discipline, pallet spacing, return air paths, loading density, preventive maintenance, backup power and refrigerant leakage. A set point is not evidence that the load stayed within specification.

Temperature heterogeneity is a persistent problem. Reviews document spatial differences within rooms, containers, pallets and packages, with consequences for residual shelf life (Jedermann et al., 2014, 2017; Badia-Melis et al., 2018). Validation therefore requires temperature mapping under representative loads and seasons, followed by risk-based sensor placement. The warmest location is not necessarily fixed; door operations, airflow obstruction and mixed loads can shift it.

Transport planning must incorporate the fact that many refrigerated vehicles are designed to maintain, not rapidly reduce, product temperature. Pre-trip inspection, equipment pull-down, verified product temperature before loading, airflow channels, door-opening control and response procedures are operational prerequisites. Time–temperature abuse is a cumulative exposure problem, but the biological response is commodity and hazard-specific; simple “minutes out of range” rules may be too crude (Ndraha et al., 2018).

4.3 Modified and controlled atmospheres

Modified-atmosphere packaging (MAP) alters oxygen, carbon dioxide and water-vapor conditions through film permeability, produce respiration, package geometry, temperature and initial gas composition. A successful design must match respiration over the full anticipated temperature range. A package optimized at stable refrigeration can become oxygen-limited during an excursion because respiration increases more rapidly than gas transmission.

Controlled studies show that packaging and atmosphere can slow selected quality changes for specific produce. For example, a consolidated cold-chain and modified-atmosphere system was evaluated for strawberries under defined supply-chain conditions (Zhao et al., 2019), and dynamic controlled atmospheres have been studied for apple quality (Mditshwa et al., 2018). These results cannot be generalized without the commodity, cultivar, maturity, film, gas, temperature, relative humidity and endpoint. Microbial consequences must also be evaluated: suppressing visible spoilage is not equivalent to controlling pathogens (Sandhya, 2010; Caleb et al., 2013). Representative evidence and the limit of each supported claim are compared in Table 1.

Table 1. Representative evidence and the limit of the supported claim

Source and design	Commodity/context	Intervention or tool	Comparator and endpoint	Supported inference	Readiness / main limitation
Duan et al. (2020)	Fruits and vegetables	Precooling methods	Cooling rate, method fit and quality across published studies	Rapid removal of field heat is foundational; method must match commodity and package	R3–R4 for established methods; heterogeneous reporting and economics
Zhao et al. (2019)	Strawberry	Refrigeration plus MAP	Defined package/cold-chain treatments; quality and shelf-life measures	Combined atmosphere and temperature control can retain strawberry quality under studied conditions	R2; cultivar, package and route specificity
Lamberty and Kreyenschmiet al. (2022)	Fresh fruit and vegetables	IoT-enabled ambient monitoring	Temperature and logistics data during supply-chain use	Feasibility of real-time condition visibility	R3 for monitoring; no controlled food-loss endpoint
Gillespie et al. (2023)	Refrigerated deliveries	IoT anomaly detection and alerts	Detection/notification performance during transport	Anomalies can be detected and communicated in a small fleet	R3 for alerts; not evidence of quantified spoilage reduction
Torres-Sánchez et al. (2020)	Fruit and vegetables	Real-time monitoring and shelf-life estimation	Sensor inputs and estimated remaining life	Temperature histories can support modeled shelf-life decisions	R2–R3; model validity is commodity- and endpoint-dependent
Defraeye et al. (2021)	Postharvest supply chains	Digital twins	Published models and prospective applications	Digital twins can integrate measurements and models for prediction	R1–R2 for most uses; validation, uncertainty and transferability gaps

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Kayikci et al. (2022)	Food supply chains/emerging economies	Blockchain framework	People, process, performance and technology barriers	Blockchain may improve agreed-record traceability when governance is present	R1–R3 for traceability; oracle, interoperability and adoption constraints

The pathway from exposure measurement to corrective action and accountable outcomes is summarized in Fig. 1.

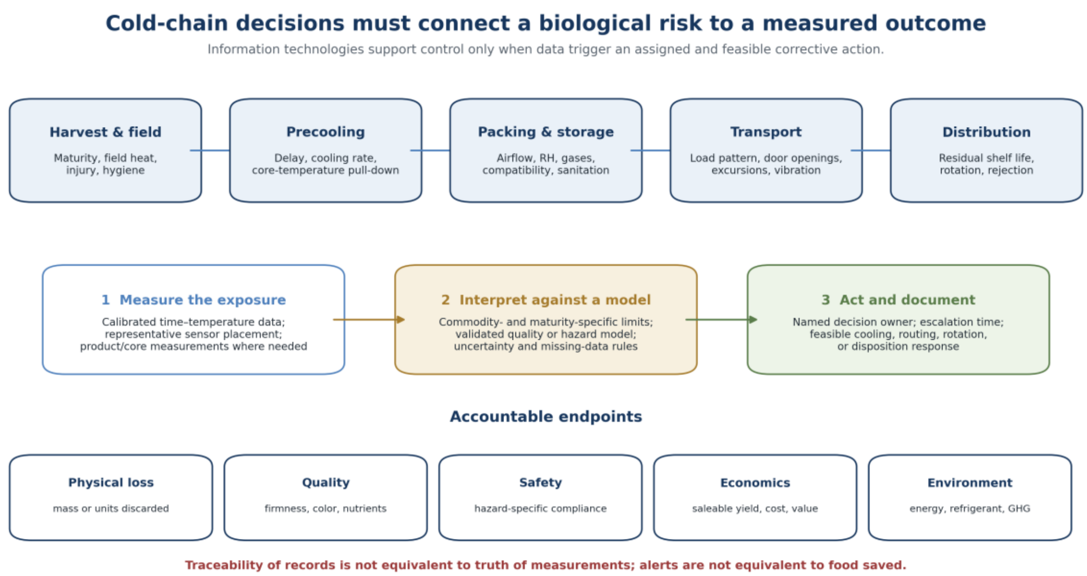

Figure 2: Cold-chain decision pathway linking supply-chain stage, measurement, interpretation, corrective action and accountable endpoints

5. Digital monitoring, traceability and prediction

5.1 Sensors and IoT: measurement before analytics

Sensors and IoT platforms can increase visibility by collecting temperature, humidity, gas, location, shock or door-status data and communicating alerts (Ben-Daya et al., 2021). Their value depends on metrological and operational details often omitted from high-level discussions. Minimum specifications include accuracy over the relevant range, calibration traceability and interval, drift, response time, resolution, sampling frequency, clock synchronization, ingress protection, battery life, memory, transmission coverage and data-recovery behavior. Installation should be validated after cleaning, maintenance or load-pattern changes.

Placement is an inference problem. A single wall sensor can describe equipment control but not the distribution of product exposure. Pallet level sensors improve spatial resolution yet still may miss hot spots or core temperatures. A risk-based design maps the chamber or route, selects sentinel locations, and states what population the readings represent. Missing data, delayed transmission, false alerts and alert fatigue require explicit rules. The receiving operator needs an escalation window and feasible response repair, reicing, rerouting, accelerated rotation, segregated inspection or disposition, otherwise real time data become retrospective documentation.

The distinction between monitoring and outcome is illustrated by Gillespie et al. (2023): the study demonstrated real-time anomaly detection and notification in three delivery vans. It supports feasibility of small-fleet monitoring and alerts, not a quantified reduction in spoilage. Similarly, remote cold-store systems can demonstrate environmental visibility (Afreen and Bajwa, 2021), but food saved must be measured with a comparator, denominator and time horizon.

5.2 Identification, sensing and trustworthy traceability

Radio-frequency identification (RFID) is primarily an identification and data-link technology; some tags incorporate sensors, but an identity tag alone does not measure temperature. Traceability architecture must define identifiers for lots, packages, pallets and locations; event semantics; time synchronization; chain of custody; data retention; and integration with inventory, quality and food safety systems. Interoperability failures can create complete records within one organization and gaps at handovers.

Blockchain can make agreed records difficult to alter after entry, but cannot verify the truth of a sensor, manual observation or certificate at the moment of entry. This is the “oracle” problem. Governance questions include who can write and correct data, how commercial confidentiality is protected, which events are mandatory, how sensor identities and calibrations are authenticated, how off-chain data are retained, and who bears cyber and recovery costs. Reviews and implementation interviews show both traceability opportunities and people/process impediments (Kamilaris et al., 2019; Kayikci et al., 2022). The supported conclusion is conditional: distributed ledgers may strengthen traceability when data governance and interoperable processes are already credible.

5.3 Prediction, AI and digital twins

A shelf-life model maps exposure and product attributes to a defined quality or safety endpoint. Required inputs may include time–temperature history, gas composition, humidity, maturity, initial quality, microbial status or handling events. Model performance should be reported on independent data from representative seasons, cultivars and routes, with calibration and discrimination metrics, uncertainty intervals and failure conditions. A black-box prediction without external validation can rank loads incorrectly when the operating distribution changes.

Digital twins extend this idea by maintaining a virtual representation of a product, package, load, equipment item or network. They can combine sensors with heat-transfer, airflow, respiration, quality-decay or logistics models to estimate unmeasured states and evaluate alternatives (Defraeye et al., 2021). Yet the twin inherits error from sensors, boundary conditions, model structure and updating rules. A high-resolution visualization is not evidence of high predictive

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validity. Deployment therefore requires version control, uncertainty propagation, explainable decision thresholds, cybersecurity and a process for retiring a model that no longer performs.

6. Optimization as a decision discipline

6.1 Formulation classes are not solution methods

Optimization selects decisions that improve one or more objectives subject to constraints. Facility location, capacity, production, inventory, allocation, routing, scheduling, temperature set-points, packaging and product disposition can be modeled at strategic, tactical or operational horizons. A linear or mixed integer formulation describes relationships among variables; stochastic, robust or fuzzy approaches represent different kinds of uncertainty. Genetic algorithms, ant colony methods, tabu search and non-dominated sorting are solution methods. Treating "fuzzy," "genetic" and "multi-objective" as equivalent optimization types obscures what decision is being made and what is guaranteed.

A reproducible model report should specify commodity and biological assumptions; geography and network; time horizon; decision variables; objectives with units; constraints; temperature or quality-decay function; uncertainty distribution or ambiguity set; data source; solver or algorithm, settings and stopping rule; instance size and computing time; baseline; sensitivity or stress tests; and validation. For multi-objective models, a single weighted sum can hide trade-offs. Pareto-efficient alternatives and the consequence of changing weights are more informative to decision-makers.

6.2 Deterioration and uncertainty

Perishability is sometimes represented only by a fixed shelf-life constraint. More informative models connect quality to time and temperature, allow heterogeneous initial quality, and restrict service or acceptance by remaining shelf life. Rong et al. (2011) integrated product quality in production and distribution decisions, while de Keizer et al. (2017) modeled heterogeneous quality decay in perishable logistics network design. Such formulations improve biological relevance but depend on the validity of decay parameters and the availability of initial-condition data.

Uncertainty arises from demand, travel time, ambient conditions, equipment failure, harvest volume and quality, energy prices and market acceptance. Scenario-based stochastic programming is appropriate when defensible probabilities exist; robust optimization protects against a defined uncertainty set; fuzzy models can represent imprecise preferences or parameters but should not substitute for missing empirical data. Every approach should report the price of robustness or recourse and compare against a deterministic baseline.

6.3 What the current model evidence supports

Bortolini et al. (2016) formulated a three-objective fresh-food distribution problem spanning cost, delivery time and carbon footprint. It demonstrates how conflicting objectives can be made explicit. Wang et al. (2021) considered collaborative distribution centers, resource sharing and temperature-control constraints in a bi-objective mixed-integer model, using a hybrid clustering/metaheuristic procedure and case/benchmark evaluation. Mejjaouli and Babiceanu

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(2018) developed a real-time rerouting architecture for cold supply chains. These studies support model feasibility and prospective improvements relative to their selected baselines; they do not establish that the same percentages will occur in another country, commodity, fleet or commercial season.

The recent systematic review by Iyer and Robb (2025) mapped 234 cold-chain optimization publications and found a broad, growing literature. Its breadth strengthens the case for optimization as an analytical core, while also exposing fragmentation in objectives, data and validation. The evidence gap is no longer a lack of algorithms; it is the limited transition from formulation and simulation to transparent, independently evaluated operational use.

Representative optimization studies and the conditions required for interpreting their results are compared in Table 2.

Table 2. Reporting and validation of representative optimization studies

Source	Problem / formulation	Decisions and objectives	Deterioration / uncertainty	Solution and validation	Transfer limitation
Rong et al. (2011)	Integrated production–distribution model	Production and distribution decisions; cost with product quality	Time/temperature-related quality considerations	Mathematical optimization with illustrative/case evaluation	Quality parameters and network assumptions require local validation
Bortolini et al. (2016)	Three-objective fresh-food distribution	Minimize cost, delivery time and carbon footprint	Perishability /time constraints; scenario-specific inputs	Multi-objective analysis with case application	Environmental result depends on boundary, fleet and demand data
de Keizer et al. (2017)	Perishable logistics network design	Facility/network flows, cost and delivered quality	Heterogeneous quality decay	Optimization with numerical/case analysis and sensitivity	Model quality depends on initial-quality and decay estimates
Mejjaouli and Babiceanu (2018)	Real-time cold-chain rerouting	Routing response to monitored conditions; logistics performance	Dynamic conditions and event data	System architecture and computational/case evaluation	Operational latency, communications and food outcome need field confirmation

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Wang et al. (2021)	Bi-objective collaborative distribution network	Resource sharing, centers/routes, cost and vehicle use	Temperature-control constraints; case parameters	MILP; clustering plus TS–NSGA-II; Chongqing case and benchmarks	Metaheuristic solutions and case data do not guarantee external performance
Wang et al. (2024)	Data-supported cold-chain cost optimization	System cost decisions using clustered data	Dataset-dependent representation	Four simulated datasets and one real dataset; computational comparison	Field loss, quality and implementation outcomes were not established
Iyer and Robb (2025)	Systematic review of optimization models	Taxonomy across cold-chain decisions and objectives	Maps treatment across 234 publications	Systematic literature synthesis	Review-level evidence; study quality and validation remain heterogeneous

These reporting requirements are organized as a staged validation pathway in Fig. 3.

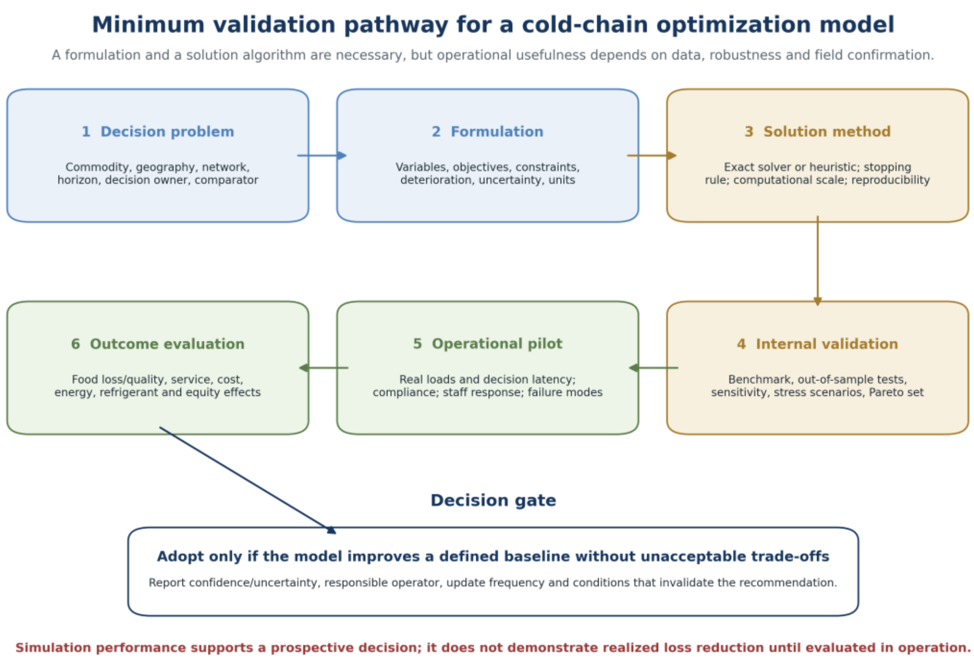

Figure 2: Minimum validation pathway for cold-chain optimization

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7. Sustainability, economics and infrastructure

7.1 Evaluate food saved and the burden of saving it

Cooling can avoid the upstream environmental burdens embodied in lost produce, but refrigeration also consumes energy, can leak high-global-warming-potential refrigerants, requires equipment and packaging, and may shift demand to a carbon-intensive grid (James and James, 2010; FAO and UNEP, 2022). The correct environmental question is not whether cooling uses energy; it is whether the intervention reduces total impact relative to a specified baseline for the same delivered function.

Life-cycle assessment should state the functional unit (for example, one kilogram of acceptable produce delivered at retail entry), system boundary, geography, season, baseline chain, grid mix, refrigeration efficiency, refrigerant type and leakage, equipment utilization and lifetime, packaging, water use, avoided loss rate and end-of-life treatment. Read et al. (2020) showed through an environmentally extended input–output analysis that the benefit of reducing food loss and waste varies by commodity, stage and impact category. Therefore, a universal statement that an intervention cuts energy or emissions by a fixed percentage is not defensible.

Sensitivity analysis is essential because modest changes in assumed avoided loss, throughput, grid carbon intensity or leakage can change the result. Rebound should also be considered: lower unit cost or longer shelf life may increase volumes, distance or inventory and offset part of the gain.

7.2 Financial viability and equitable access

A technically effective intervention can fail if cash flow, utilization or service arrangements are weak. Financial assessment should separate capital expenditure, installation, land and grid connection from electricity or fuel, labor, calibration, data subscription, refrigerant, packaging, sanitation, maintenance, spare parts and downtime. The relevant denominator may be crates or tonnes processed, saleable kilograms delivered, or avoided rejection not nominal equipment capacity.

For shared infrastructure, the business case depends on seasonal throughput, scheduling among growers, payment collection, product aggregation and assured market access. Smallholders may face barriers even where total-chain savings are positive because costs and benefits accrue to different actors. Contracts, cooperatives, pay-per-crate services, leasing, concessional finance, insurance and public private facilities can redistribute risk, but require transparent service levels and governance.

Skills and spares are part of the technology. A minimum implementation plan covers operator training, preventive maintenance, calibration, sanitation, emergency procedures, backup power, refrigerant service, data ownership and vendor exit. In unreliable-grid contexts, passive insulation, evaporative cooling where climatically suitable, thermal storage, solar-assisted systems and coordinated daytime precooling may be more robust than an oversized conventional plant. Options should be compared on delivered service, not equipment label.

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7.3 Readiness-based adoption matrix

The resulting evidence- and readiness-based adoption gates are summarized in Table 3.

Table 3. Evidence- and readiness-based implementation matrix

Intervention	Best-fit problem	Minimum data and controls	Evidence readiness	Main failure mode	Adoption gate
Shade, shorter field delay, gentle handling	Field heat, water loss, bruising	Harvest time, initial temperature, delay, injury/rejection	R4 in principle; local outcome varies	No coordination or containers that cause injury	Measured reduction in delay/injury against current practice
Commodity-matched precooling	High field heat and rapid deterioration	Initial/core temperature, target, pull-down time, package/airflow, hygiene	R3–R4	Method–commodity mismatch, slow/uneven cooling, contaminated water	Reproducible core-temperature pull-down and quality benefit at viable throughput
Cold storage and refrigerated transport	Maintain condition after cooling	Temperature map, product temperature, capacity, airflow, maintenance, backup	R4 for basic control; route-specific design	Warm loading, blocked airflow, doors, outages, leakage	Stable product exposure plus saleable-yield/service improvement
MAP / controlled atmosphere	Respiration, moisture and gas management	Commodity/cultivar, maturity, respiration, film permeability, gas/RH, temperature	R2–R3 for many applications	Anaerobiosis, condensation, injury or microbial misconception	Quality endpoint improved without safety or sensory penalty under excursion tests
IoT condition monitoring	Invisible excursions and delayed response	Calibrated sensors, representative placement, connectivity, alert ownership	R3 for monitoring	Drift, missing data, false alerts, no feasible action	Alerts are timely, acted upon and linked prospectively to a measured endpoint

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RFID / blockchain traceability	Lot identity and fragmented records	Data standards, identity, custody, permissions, correction and audit rules	R2–R3 for traceability	"Garbage in, immutable garbage out"; interoperability/cost	Faster or more complete traceability against baseline with verified data governance
Shelf-life model / digital twin	Rotation, routing and scenario testing	Initial quality, exposure data, validated model, uncertainty and update rules	R1–R3	Dataset shift, unvalidated endpoint, false precision	Independent validation and decision benefit exceeding error/cost
Network, inventory or routing optimization	Multi-actor trade-offs and constrained resources	Audited demand, time, cost, capacity, quality-decay and uncertainty data	R2; limited R3–R4 outcome evidence	Unrealistic parameters, opaque weights, no operational ownership	Better baseline performance in stress tests followed by a monitored field pilot

8. Prevention, redistribution and circular routing

Circular-economy language can obscure the priority of preventing edible food from becoming surplus or waste. A food-use hierarchy places prevention first, followed by redistribution for human consumption where quality, safety and logistics permit; conversion to animal feed under applicable controls; material or biochemical valorization; energy recovery; composting; and disposal (Papargyropoulou et al., 2014; Teigiserova et al., 2020). These pathways are not environmentally or nutritionally equivalent.

Cold-chain information can support earlier rotation, transfer or donation by estimating remaining quality life, but dynamic discounting or redistribution must not pressure staff to sell or donate unsafe food. Disposition rules should use validated criteria, preserve traceability and identify who accepts liability and transport cost. Product rejected for cosmetic quality may be suitable for another human-food outlet; product with uncertain contamination or temperature history may not be.

Valorization decisions require mass balance, safety, market demand and life-cycle assessment. A technically feasible extract, feed or bioenergy pathway may consume more energy, water or chemicals than a simpler route, or may depend on a seasonal feedstock too variable for reliable operation. Optimization of reverse flows should therefore report the amount and condition of material, distance, yield, displaced product, processing burden and final market—not simply "waste minimized."

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9. Comparative synthesis and decision framework

The evidence supports a staged investment logic. Firstly, stabilize biological and operational control. Specify commodity, maturity, intended market life and acceptance endpoint. Reduce field delay and injury; match precooling to the product and package; verify core pull-down; map storage and transport; maintain hygiene, airflow and equipment; and assign operational responsibility. Secondly, instrument the highest-consequence uncertainties includes selection of sensors and sampling frequency from a decision need rather than a desire for data volume. Map heterogeneity before choosing locations. Establish calibration, missing-data and escalation procedures, and verify that the responsible person can act within the remaining response window.

Thirdly we validate and interpret the data. Connect exposures to commodity-specific quality or hazard models. Report uncertainty, independent validation and conditions that invalidate the model. Identification and traceability data should be interoperable and governed, with clear custody and correction rights. Fourth, optimize defined decisions. Build a baseline and specify variables, objectives, constraints and units. Include quality decay or remaining shelf life where it changes decisions. Compare exact and heuristic solutions appropriately, report computational scale and sensitivity, and show Pareto trade-offs rather than hiding them in unexamined weights.

Fifth, verify outcomes in operation. A monitored pilot should measure physical or quality loss, service, cost, energy and unintended effects against the current practice over enough loads and seasons to expose failure modes. The model or technology should be revised or withdrawn if performance shifts outside the validated range.

This sequence does not imply that every chain requires blockchain, AI or a digital twin. A low-cost improvement in field delay or airflow may produce a larger, more certain benefit than an advanced platform. Conversely, a high-volume, multi-route chain with stable physical control and reliable data may benefit substantially from dynamic routing or shelf-life-aware inventory. Readiness is therefore a property of the technology–commodity–decision–context combination.

10. Limitations and research priorities

This review has four limitations. First, it is a structured narrative synthesis without prospective registration or retained screening counts, so selection bias and incomplete retrieval are possible. Second, terminology and endpoints vary widely; many studies report temperature, model cost or nominal shelf life rather than physical loss, saleable yield or independent safety outcomes. Third, laboratory packages, single-route pilots and modeled networks have limited external validity across cultivars, seasons, countries and infrastructure. Fourth, English-language and accessible-source restrictions may underrepresent local operational knowledge in high-loss regions.

Research should address the following priorities includes reporting of load count, mass denominator, baseline, product/core temperature, rejection, quality, safety, cost and energy over multiple seasons. Publish sensor calibration, drift, placement rationale, sampling, missingness and mapping of pallets, vehicles and rooms. Include initial quality, maturity and uncertainty; validate on independent routes and state failure boundaries. Release de-identified instances or generators, parameter sources, code or pseudocode, solver settings, stopping rules and full baseline/sensitivity results.

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Move beyond simulation to prospective pilots that measure whether recommendations were implemented and whether food, cost or emissions changed. Integrate avoided food loss with electricity, fuel, refrigerant leakage, packaging, water, maintenance, capital, service and equity. Compare modular, shared and passive solutions; analyze financing, utilization, technical service, gender and bargaining effects. Validate hazard-specific decision rules and prevent quality predictions from being used as unqualified safety assurances.

11. Conclusion

Cold-chain optimization for fresh horticultural produce is not principally a contest among technologies. It is the disciplined connection of a commodity-specific deterioration mechanism to a feasible control action, a representative measurement, a validated interpretation and an accountable outcome. The strongest practical evidence supports rapid and appropriate removal of field heat, stable temperature and humidity, hygienic handling, airflow and package compatibility. These controls should be established before advanced analytics are expected to compensate for basic operational failure.

Digital monitoring can reveal excursions; RFID can identify lots; blockchain can preserve agreed records; predictive models and digital twins can estimate unmeasured states; and optimization can compare constrained alternatives. None independently guarantees temperature control, data truth, corrective action, microbiological safety or food saved. Current optimization evidence is rich in formulations and simulations but thinner in replicated commercial outcome evaluation.

Investment should therefore be gated by biological fit, data quality, operator ownership, baseline comparison, uncertainty, total cost and environmental burden. Promising tools should advance from laboratory or simulation evidence to operational pilots and then to replicated outcome studies. When studies report commodity, comparator, units, validation scale and trade-offs transparently, cold-chain optimization can become a credible route to greater saleable yield, safer decisions and lower whole-system impact rather than an unsupported technological promise.

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Statements and Declarations

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Postharvest Processing and Storage Technologies for Horticultural Produce and Grains: Evidence, Trade-offs and Strategies for Loss Reduction

Muhammad Usama Iqbal Awan*[1]

[1]Nanjing Agricultural University

*Corresponding Author: njaufst@gmail.com

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Abstract

Postharvest interventions can preserve food already produced, but their performance depends on commodity physiology, initial quality, operating conditions, and supply-chain capability. This structured narrative review asks how drying, hermetic storage, pre-cooling and cold-chain management, controlled or modified atmospheres, and selected active or digital technologies should be matched to horticultural produce and grains when efficacy, quality, safety, cost, energy, and implementation constraints are considered together. Peer-reviewed and authoritative evidence published mainly from 2000 to 22 July 2026 was searched and appraised; older landmark sources were retained where they define established principles. Current global indicators show that 13.3% of food is lost between production and the stage immediately before retail, while consumer-facing waste is a separate problem. No universal loss percentage or shelf-life multiplier is supported across commodities. Drying and hermetic storage are comparatively accessible for grains, provided that safe initial moisture, package integrity, and re-opening practices are controlled. For fresh produce, rapid removal of field heat and an unbroken, commodity-specific cold chain remain the foundation; controlled atmosphere, modified atmosphere packaging, 1-methylcyclopropene, coatings, or ozone are adjuncts rather than substitutes. Nano-enabled packaging and digital twins remain conditionally promising because migration, validation, interoperability, and regulatory questions persist. Two comparative matrices translate the evidence into a selection framework. The strongest strategy is therefore not the most complex technology, but a validated system whose physiological fit, hazard controls, resource demands, and market value are aligned.

Keywords: Food loss; Postharvest; Cold chain; Hermetic storage; Modified atmosphere packaging; Drying; Technology selection

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1. Introduction

Harvest does not stop biological change. Fruit and vegetables continue to respire and transpire; many also synthesize or respond to ethylene. Grains and pulses are less metabolically active after adequate drying, but remain vulnerable to moisture migration, insects, rodents, fungi, and mechanical damage. These processes can reduce mass, nutritional or sensory quality, safety, market grade, and economic value. They are related outcomes, not interchangeable measures.

The contemporary baseline is more precise than the often-repeated claim that one third of all food is “lost after harvest.” The Food and Agriculture Organization of the United Nations (FAO) estimates that 13.3% of food was lost globally in 2023 after production and before retail; the indicator varies by region and commodity group (FAO, 2026). The United Nations Environment Programme estimated 1.05 billion tonnes of food waste at retail, food-service, and household stages in 2022, including inedible parts (UNEP, 2024). These two indicators cover different supply-chain boundaries, denominators, and measurement systems. The older 1.3-billion-tonne estimate combined loss and waste across stages and should not be used as a current postharvest-loss statistic (FAO, 2019).

“Postharvest loss” is used here for a measurable reduction in the quantity, quality, safety, or economic value of crops from harvest through handling, storage, transport, processing, and wholesale, before retail. “Food loss” follows the FAO boundary from postharvest production to, but excluding, retail. “Food waste” refers to retail and consumption stages. Quantitative loss is a reduction in edible mass or count; qualitative loss includes deterioration of safety, nutrient content, sensory properties, processing functionality, or grade. Economic loss additionally depends on price, timing, and market acceptance. Shelf life is the time during which a product remains safe and acceptable under stated conditions and against defined end points. Consequently, shelf-life extension is meaningful only when the control, temperature, humidity or atmosphere, package, duration, and rejection criteria are reported.

Loss estimates are heterogeneous because field sampling, visual grading, farmer recall, mass balance, and economic valuation produce different answers. A meta-analysis of postharvest studies in sub-Saharan Africa demonstrated wide variation across commodities, stages, and measurement approaches (Affognon et al., 2015). Broad percentages detached from these

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conditions can misdirect investment. Technology assessment likewise fails when it reports a biological effect but omits energy reliability, sanitation, package integrity, operator skill, worker exposure, waste management, or market value.

This review is restricted to horticultural produce (whole and fresh-cut fruit and vegetables) and grains or pulses from harvest to pre-retail distribution. Fish, meat, dairy products, and primary crop production are outside scope. The technologies considered are drying, ambient and evaporative systems, hermetic storage, pre-cooling and cold-chain management, controlled atmosphere (CA), modified atmosphere packaging (MAP), 1-methylcyclopropene (1-MCP), edible coatings and essential oils, ozone, nano-enabled packaging, and sensor-supported decision tools. The geographical perspective is global, with explicit attention to resource-constrained supply chains.

The review question is: How should postharvest processing and storage technologies be selected for horticultural produce and grains when evidence of loss control is weighed against quality, safety, energy, cost, infrastructure, and operational risk? The novelty is a decision framework that separates established principles from transferable but condition-dependent evidence and emerging applications, while comparing lower-cost and advanced systems on the same criteria.

2. Review Approach

A structured narrative search was conducted for English-language evidence published principally from January 2000 through 22 July 2026. Search groups combined *postharvest loss*, *food loss*, *shelf life*, *quality*, or *mycotoxin* with commodity terms (fruit, vegetable, grain, cereal, pulse, apple, strawberry, maize) and technology terms (drying, solar drying, water activity, hermetic, pre-cooling, cold chain, controlled atmosphere, modified atmosphere packaging, 1-MCP, chitosan, essential oil, ozone, nanomaterial, sensor, digital twin). PubMed and publisher platforms were used to identify peer-reviewed records; Crossref, DOI, PubMed, and publisher metadata were used to verify bibliographic details. Authoritative FAO, UNEP, USDA, FDA, EFSA, Codex Alimentarius, and European Union repositories were searched for indicators, commodity guidance, and regulatory or safety documents. Reference lists were searched for landmark studies.

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Eligible sources addressed edible horticultural produce or grains after harvest and reported a mechanism, operating condition, outcome, implementation factor, or regulatory requirement relevant to the review question. Preharvest-only studies, non-crop foods, non-traceable records, duplicated references, and sources whose title or content did not support the cited claim were excluded. Primary experiments were preferred for numerical treatment effects. Reviews and institutional reports were used for established principles, synthesis, and policy context. Evidence was appraised as: (i) established, when widely commercialized or supported by consistent operational evidence; (ii) transferable with conditions, when performance depends strongly on commodity and system design; or (iii) emerging, when evidence is mainly laboratory or pilot scale or regulatory uncertainty remains. Because treatment conditions and end points were highly heterogeneous, no pooled effect or universal shelf-life multiplier was calculated.

3. Assessment of Losses

3.1 Horticultural Produce

Field heat is the sensible heat present in a commodity at harvest. It is distinct from heat gained later through solar exposure, warm loading areas, vehicle walls, respiration, or refrigeration failure. Delayed shade and cooling increase respiration and water loss; rough harvest and repeated transfers create bruises, abrasions, and compression damage. Contaminated wash water can spread microorganisms across a lot. In storage and distribution, temperature excursions, poor airflow, over-stacking, incompatible mixed loads, and condensation may become more important than the nominal set point.

Respiration and ethylene responses differ markedly among commodities and cultivars. Low temperature usually slows deterioration, but the safe temperature cannot be expressed as a generic 0-10 °C range. Temperate fruit may tolerate temperatures near 0 °C, whereas banana, mango, papaya, cucumber, eggplant, and some tomato maturity stages can develop chilling injury at low but non-freezing temperatures. The USDA commercial-storage handbook therefore specifies commodity-dependent temperature, relative humidity, expected storage duration, and physiological hazards (Gross et al., 2016). A measurement plan should record pulp or product temperature rather than air temperature alone, time to the target temperature, relative humidity, weight loss, decay, firmness or texture, color, flavor or volatile changes, and market rejection.

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3.2 Grains and Pulses

For grains, the principal control variables are initial moisture content, water activity (a_v), temperature, oxygen transfer, insect pressure, sanitation, and the water-vapor barrier of the store. Moisture content expresses water relative to product mass, whereas a_v indicates the thermodynamic availability of water and is related to equilibrium relative humidity. The relationship is product- and temperature-specific through the sorption isotherm. Thus, a single moisture content below 10-12% or an a_v below 0.3 cannot "ensure stability" for all grains, seeds, storage durations, or quality end points. Regulatory or procurement limits for a commodity are not interchangeable with a mechanistic a_v threshold (FDA, 2014).

Dry storage can prevent or slow further fungal growth and mycotoxin formation; it does not remove aflatoxin or other toxins already present. Sampling error is also important because mycotoxins can be highly heterogeneous within a lot. Appropriate outcomes include dry-matter loss, insect count, damaged kernels, germination, mold incidence, toxin concentration, price discount, and rejection; not simply visible weight loss.

3.3 A Minimum Reporting Set

Claims about effectiveness should identify the commodity and cultivar, harvest maturity and initial quality, intervention and dose, comparator, package or storage geometry, product load, temperature and relative humidity, O_2/CO_2 conditions where relevant, duration, replication, and pre-defined safety and quality end points. For field adoption, the unit of analysis should include rejected loads, downtime, maintenance, consumables, labor, energy and water use, and net value recovered. This reporting set makes biological efficacy comparable to operational performance.

4. Technology Evidence and Boundary Conditions

4.1 Drying: Control Water, Heat, Contamination, and Rewetting

Drying reduces water availability and mass for transport, but it can also cause case hardening, color change, flavor loss, vitamin degradation, lipid oxidation, and poor rehydration. Open sun drying is inexpensive and accessible but exposes food to dust, animals, insects, rainfall, uneven heating, and nighttime moisture uptake. Enclosed solar dryers improve hygiene and heat capture; their output still varies with solar radiation, load depth, airflow, collector area, and weather. Hot-air dryers provide greater control but require reliable energy. Heat-pump, infrared, microwave,

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radio-frequency, freeze, and hybrid drying can improve rate or selected quality attributes, but capital, maintenance, throughput, and non-uniform heating become decisive (Ratti, 2001; Onwude et al., 2016; Devan et al., 2020; Kong et al., 2024).

Drying should therefore be designed backwards from a product-specific safe-storage target. Moisture content and a_v should be measured after temperature equilibration, and the package must limit water-vapor transfer during the intended climate and storage time. A fast-drying rate is not automatically optimal: surface sealing can trap internal moisture, while excessive temperature may damage seed viability or heat-sensitive nutrients. Model-based drying curves are useful only after validation at the relevant bed depth and airflow (Onwude et al., 2016).

Direct-fire and smoke drying require an additional chemical-hazard assessment. Incomplete combustion and direct contact with smoke can deposit polycyclic aromatic hydrocarbons (PAHs). Codex recommends controlling fuel type and moisture, combustion conditions, distance from the heat source, smoke contact, fat dripping, and equipment cleanliness (Codex Alimentarius Commission, 2009). Applicable contaminant limits must be verified in the destination market; for example, European Union limits are consolidated in Regulation (EU) 2023/915 (European Commission, 2023). Smoke should not be treated as a free preservative benefit without measuring PAHs and sensory acceptability.

4.2 Ambient, Evaporative, and Hermetic Storage

Shade, reflective roofs, ventilated stacks, raised pallets, clean containers, and first-in-first-out rotation are low-cost controls. Evaporative cooling can lower temperature when the air is dry enough, but cooling potential diminishes as ambient humidity rises. It consumes water, raises local relative humidity, and does not provide a fixed product temperature. It is most suitable for commodities that benefit from high relative humidity and tolerate surface moisture; sanitation and drainage remain necessary.

Hermetic bags or silos restrict gas exchange. They do not instantly eliminate oxygen. O_2 declines and CO_2 rises progressively through respiration by insects, grain, and microorganisms; the trajectory depends on seal integrity, liner permeability, infestation, grain moisture, temperature, fill ratio, and duration. In an eight-month maize study, PICS bags buffered internal humidity and limited insect damage, but oxygen patterns differed between infested and non-infested bags

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(Williams et al., 2017). Opening a hermetic bag renews oxygen and moisture exchange; repeated opening has been associated with fungal growth and aflatoxin risk under unfavorable grain conditions (Tubbs et al., 2016).

Hermetic storage is therefore an insect-management and moisture-buffering tool, not a substitute for cleaning and drying. Grain must enter at a locally validated safe moisture content, liners must be inspected for punctures, and repeated retail withdrawal should use smaller units or controlled decanting. Economic value depends on bag life, counterfeit avoidance, training, harvest-time credit, and the ability to sell when prices improve. Cross-country analysis indicates that adoption and greenhouse-gas implications vary with baseline loss, reuse, and market conditions (Dijkink et al., 2022).

4.3 Pre-cooling and the Cold Chain

For most fresh produce, rapid removal of field heat followed by stable temperature management has the widest technical foundation. Room cooling is simple but often slow. Forced-air cooling draws cold air through packages and requires aligned vents, controlled pressure, and loading patterns that prevent short-circuiting. Hydro-cooling can be fast for water-tolerant commodities, but water sanitation, infiltration, disposal, and package wet strength must be managed. Vacuum cooling is especially effective for leafy products with high surface area, although evaporative mass loss and high equipment cost require water make-up and adequate throughput. Icing is limited to commodities and packages that tolerate direct water and near-0 °C conditions (Gross et al., 2016; Duan et al., 2020; Mercier et al., 2017).

Pre-cooling method selection must consider cooling rate, cultivar, package geometry, water sensitivity, sanitation, energy and water availability, and the next distribution step. A cold room cannot correct warm centers rapidly if airflow paths are blocked. Conversely, pre-cooled produce loses the benefit if it waits on an unrefrigerated dock. Time-temperature integration is more informative than a single dispatch reading because deterioration is cumulative and spatially variable (Mercier et al., 2017). Sensors should be positioned at anticipated warm points, calibrated, and linked to corrective action.

A primary strawberry supply-chain experiment illustrates both synergy and specificity. Zhao et al. combined an insulated expanded-polystyrene package, phase-change cooling, and MAP and

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found better appearance, firmness, soluble solids, color, and respiration control than the study control under the tested simulated distribution conditions (Zhao et al., 2019). This result does not establish a universal multiplier for strawberries: package size, film, initial fruit condition, atmosphere, season, transport profile, and rejection criterion govern transferability.

4.4 Controlled and Modified Atmospheres

CA storage actively measures and controls room gases; dynamic CA adjusts the low-O_2 set point in response to fruit status. MAP modifies the package headspace through gas flushing or commodity respiration and film transfer. Vacuum packaging is a distinct process and should not be used as a synonym for gas-flush MAP. For passive MAP, the eventual atmosphere emerges from the balance among respiration rate, film O_2 and CO_2 permeability, film area and thickness, perforations, fill weight, free headspace, seal quality, and temperature. Water-vapor transfer and condensation must be considered alongside gases (Sandhya, 2010; Caleb et al., 2013).

Lower O_2 and elevated CO_2 can slow respiration, ethylene action, and some spoilage organisms, but the response is commodity-, cultivar-, maturity-, temperature-, and duration-specific. Excessive CO_2 can cause tissue injury, browning, or off-flavors. O_2 below the commodity tolerance can induce fermentative metabolism, ethanol and acetaldehyde accumulation, loss of flavor, and potential microbiological concern in minimally processed foods. The safe operating window narrows during temperature abuse because respiration can rise faster than film permeability. Reviews of apple dynamic CA emphasize the risk of low-O_2 and CO_2 injury even where firmness and scald control improve (Mditshwa et al., 2018).

Accordingly, a gas recipe cannot be transferred solely because two commodities have similar respiration rates. Validation should include continuous or repeated headspace measurement, leakage testing, sensory end points after retail simulation, and challenge or hazard assessment where reduced oxygen could favor pathogens. CA and MAP are best regarded as additions to temperature control, not replacements for refrigeration or sanitation.

4.5 4.5 1-MCP, Coatings, Essential Oils, and Ozone

1-MCP binds ethylene receptors and can delay ethylene-mediated ripening and senescence. Its effect depends on crop, cultivar, maturity, time after harvest, concentration, exposure duration, temperature, and subsequent atmosphere. Some fruit may show delayed or incomplete aroma

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development or impaired recovery of ripening. Reviews and meta-analysis confirm substantial heterogeneity among climacteric fruit and response variables (Watkins, 2006; Zhang et al., 2020). Use must follow the commodity, formulation, dose, and authorization approved in the destination jurisdiction; "1-MCP treatment" is not a single transferable process.

Chitosan and other edible coatings can reduce moisture or gas transfer and serve as carriers for antimicrobials. Performance depends on polymer molecular properties, pH, plasticizer, coating thickness, drying, adhesion, and fruit surface. Chitosan source, allergen communication, and local food-contact or additive status require attention. Essential oils can inhibit microorganisms in vitro, but release from a food matrix and the concentration required for control may produce unacceptable aroma or flavor. A recent experiment on fresh-cut 'Braeburn' apples used a 1.5% chitosan system containing acids and oregano or cinnamon-leaf essential oil during 9 d at 4 ± 1 °C; several quality and microbial indicators improved, while higher oil concentrations affected visual or sensory performance (Ali et al., 2025). This is promising laboratory evidence, not a universal commercial treatment. Broader chitosan evidence likewise remains commodity- and formulation-specific (Romanazzi et al., 2017).

Ozone is a strong oxidant applied in water or gas. Antimicrobial effect is governed by concentration × contact time, ozone demand of organic matter, humidity, temperature, water quality, mixing, surface accessibility, and organism. Excess exposure can bleach pigments, damage tissue, oxidize lipids or aroma compounds, and corrode equipment. Worker exposure, off-gas destruction, interlocks, leak detection, and validated monitoring are mandatory. Reviews report variable efficacy and tolerance across fresh and fresh-cut produce (Miller et al., 2013; Botondi et al., 2021). In the United States, ozone use as an antimicrobial in food is governed by 21 CFR §173.368 (FDA, 2001); other jurisdictions may differ. A universal 0.1-0.5 ppm recommendation is therefore inappropriate.

4.6 Nano-Enabled Packaging and Digital Tools

Nanoclays, metal or metal-oxide particles, and nano-structured biopolymers may alter barrier, mechanical, UV, antimicrobial, or indicator functions. However, "nano-packaging" describes many materials and exposure pathways. Evaluation must identify particle size distribution, surface chemistry, loading, matrix, intended function, migration under the worst foreseeable food/time/temperature conditions, oral hazard, environmental release, recycling compatibility,

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and end-of-life behavior. EFSA guidance requires specific assessment of small particles in regulated food and feed applications (EFSA., 2021). Migration is not merely theoretical: nanosilver was released from tested commercial food containers under experimental conditions (Echegoyen & Nerín, 2013). Environmental or sustainability superiority cannot be asserted without a comparative life-cycle assessment. Most nano-enabled antimicrobial applications should therefore be labeled emerging unless the exact material and intended use have regulatory clearance and scaled validation.

Time-temperature indicators, data loggers, radio-frequency identification, predictive shelf-life models, and digital twins can reveal weak points and support first-expired-first-out decisions. They do not cool food. Data value depends on sensor accuracy, calibration, sampling interval, placement, connectivity, model validity, data ownership, and a defined response when limits are exceeded. Distributed ledgers can preserve selected records but cannot prove that the original temperature measurement was accurate. Digital twins for fresh produce remain promising because they can estimate spatial and product-specific histories, but validation and interoperability are active challenges (Defraeye et al., 2021). Intelligent logistics evidence supports decision assistance rather than autonomous assurance (Jedermann et al., 2014)

5. Comparative Evidence and Selection Framework

Table 1 summarizes representative evidence and, importantly, the conditions that limit transfer. It is not a ranking of technologies. Table 2 compares implementation demands and readiness. In both tables, “widely adopted” means that commercial examples exist; it does not imply universal suitability or regulatory authorization.

Table 1. Representative evidence and boundary conditions for postharvest interventions

Interven tion	Commo dity and study context	Operating conditions and comparato r	Reported end points	Evidence/rea diness	Principal boundary conditions	Refere nces

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PICS hermetic bag	Maize; eight-month primary study	Triple-layer hermetic bags, infested/non-infested treatments; woven bags as comparators; ambient temperature varied	Internal O_2/RH, moisture, insect damage, germination	Established for insect control; transferable with training	Initial moisture, puncture/seal, infestation, temperature, bag authenticity, re-opening	(Williams et al., 2017)
Re-opening hermetic bags	Maize; experimental storage	Hermetic bags opened during storage versus sealed handling	Grain quality, fungal growth, aflatoxin	Strong warning evidence	Oxygen renewal, ambient humidity, initial contamination and moisture; toxins already present are not removed	(Tubbs et al., 2016)
Integrated cold package + MAP	Fresh strawberry; simulated supply chains	Insulated EPS package, phase-change cooling and MAP	Appearance, firmness, color, soluble solids, respiration	Pilot/transferable	Cultivar, initial quality, film and pack geometry, season, time-	(Zhao et al., 2019)

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		versus study control			temperature profile, recycling of EPS/PCM	
Pre-cooling and cold chain	Multiple horticultural commodities; technical handbook and reviews	Room, forced-air, hydro-, vacuum cooling or icing selected by commodity and package	Cooling rate, mass loss, disorders, decay, market life	Widely adopted	Chilling sensitivity, vent alignment, sanitation, water/energy, refrigerant, uninterrupted distribution	(Gross et al., 2016; Duan et al., 2020; Mercier et al., 2017)
Passive/active MAP	Fresh and fresh-cut produce; reviews	Respiring product in selected film or gas-flushed package; conventional air package	Headspace gases, respiration, texture, color, decay and microbial outcomes	Widely used but highly product-specific	Temperature abuse, permeability, fill weight, perforation, condensation, anaerobiosis and seal failure	(Sandhya, 2010; Caleb et al., 2013)
Dynamic CA	Apples; review of research and practice	Low O_2 adjusted to fruit response versus static CA	Firmness, color, scald and gas injury	Commercial for selected apples	Cultivar/maturity, sensor/control accuracy, low-O_2 and high-CO_2	(Mditshwa et al., 2018)

		or air storage			injury, operator expertise	
1-MCP	Climacteric fruit; meta-analysis and review	Formulation- and crop-specific exposures versus untreated fruit	Firmness, color, ethylene, respiration and sensory ripening	Commercial for authorized uses	Maturity, dose, exposure, temperature, cultivar, aroma/ripening recovery, jurisdiction	(Watkins, 2006; Zhang et al., 2020)
Chitosan + essential oils	Fresh-cut 'Braeburn' apple; 9 d at 4 ± 1 °C	1.5% chitosan/acid matrix with oregano or cinnamon-leaf oils versus controls	Physicochemical, antioxidant, visual and microbial indicators	Laboratory/emerging formulation	Sensory threshold, coating uniformity, oil release, chitosan source, scale-up and authorization	(Ali et al., 2025)
Ozone	Fresh/fresh-cut produce; reviews and regulation	Aqueous or gaseous treatment; dose must be validated	Surface microorganisms, decay, color, texture and oxidation	Commercial equipment; process-specific validation	C×t, ozone demand, humidity, commodity injury, worker exposure, off-gas and corrosion	Miller et al., 2013; Botondi et al., 2021; (FDA, 2014)

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Nano-enabled food contact	Commercial nanosilver containers; migration study EFSA guidance	Food simulants under defined time/temperature conditions	Particle migration and characterization	Emerging/regulated case by case	Migration, particle identity/dose, toxicity, matrix changes, environmental fate, approval and LCA	(EFSA, 2021; Echegoyen & Nerín, 2013)

Table 2. Technology selection and implementation matrix

Technology	**Best physiological or hazard fit**	**Infrastructure / utilities**	**Operator and monitoring demand**	**Cost and scalability profile**	**Resource-constrained suitability**	**Readiness / critical failure mode**
Enclosed solar or hybrid drying	Grains, pulses, sliced produce; water-activity control	Dryer, clean air, weather protection; optional backup heat	Moisture/a_v checks, loading and endpoint control	Low-moderate capital; modular; weather affects throughput	High where solar resource and maintenance exist	Established/transferable; under-drying, rewetting or overheating
Evaporative cooling	Water-tolerant produce in hot, dry air	Clean water, shade,	Wet-bulb potential,	Low capital; climate-limited	Moderate-high in arid settings	Established niche; high humidity removes

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		drainage, airflow	hygiene, daily water management			cooling capacity
Hermetic bags/silos	Dry grains/pulses; insect exclusion and modified atmosphere	Validated container and dry grain; little power	Moisture check, puncture inspection, correct sealing	Low unit capital; scalable; recurring bags/repairs	High	Widely adopted; wet grain, counterfeit/failed liner, frequent opening
Conventional cold chain	Most fresh produce at commodity-specific temperature	Pre-cooler, insulated store/vehicle, reliable power and refrigerant service	Continuous time-temperature control, airflow and calibration	High capital/operating cost; strong scale economies	Moderate where hubs, finance and service exist	Widely adopted; breaks in chain, poor loading or wrong temperature
CA storage	Long-term high-value fruit/vegetables	Gas-tight rooms, analyzers, control and backup	High skill; continuous gas and safety monitoring	High capital; requires throughput	Low for individual smallholders; possible	Commercial; leakage, gas injury, unsafe entry

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					cooperatives	
MAP	Packaged fresh/fresh-cut produce	Validated films, sealing, gas testing; often refrigeration	Medium-high design/QC demand	Moderate; scalable at packhouse	Moderate with reliable film and cold chain	Commercial; temperature-driven anaerobiosis/condensation
1-MCP	Selected ethylene-responsive, authorized crops	Sealed treatment space/formulation; cold chain often follows	Dose, maturity, exposure and authorization control	Moderate; service models can scale	Moderate for aggregated lots	Commercial for specified uses; poor ripening/aroma or non-compliance
Edible coating/essential oil	Water-loss or surface-decay control in selected produce	Food-grade formulation, application and drying	Composition, viscosity, thickness, sensory and microbiology QC	Low-moderate equipment; formulation costs	Potentially moderate	Pilot-to-commercial depending formulation; uneven coat/off-flavor

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Ozone	Surface or water sanitation adjunct	Generator, monitor, interlocks, ventilation /off-gas	High safety and process-validation on demand	Moderate-high; no stored chemical but maintenance required	Low-moderate	Commercial equipment; worker exposure or commodity oxidation
Nano-enabled packaging	Barrier/active/indicator functions for specific packs	Specialized material supply and food-contact validation	Migration, specifications, traceability and end-of-life assessment	High development/ regulatory cost	Currently low	Emerging; migration/toxicology or recycling failure
Sensors/ digital twins	Detect thermal history and support logistics decisions	Calibrated sensors, connectivity, software and response system	Data governance, model validation, corrective action	Modular sensors to high-cost integration	Moderate if decisions are actionable	Pilot-to-commercial; uncalibrated data or alerts without response

Selection should start with the dominant mechanism of loss, not a preferred device. For a dry grain lot with insects, hermetic containment after validated drying may outperform refrigeration. For a highly respiring berry, shade, rapid pre-cooling, suitable packaging, and stable distribution temperature are the foundation; adding MAP without temperature control can increase anaerobic

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risk. For a chilling-sensitive fruit, a lower set point can be worse than a slightly warmer, stable temperature. The following sequence is recommended:

1. Define commodity, cultivar, maturity, lot condition, hazard, and target market;
2. Locate the principal loss point using measured mass, grade, safety, and temperature/moisture data;
3. Specify the required storage or distribution time and acceptance end points;
4. Screen technologies for physiological compatibility and food-safety or worker-safety constraints;
5. Assess electricity, fuel, water, packaging, sanitation, maintenance, spare parts, skill, finance, and throughput;
6. Pilot against the current practice across realistic seasonal extremes; and
7. Scale only when value recovered exceeds total life-cycle cost and performance is monitored.

6. Quality, Nutrition, and Safety Trade-offs

"Quality retention" should not be represented by appearance alone. External color or firmness may remain acceptable while aroma, sweetness-acid balance, texture, vitamins, phenolics, or microbiological safety deteriorate. Conversely, processing may reduce a heat-sensitive vitamin while improving microbiological stability and year-round nutrient availability. Quality is commodity- and market-specific and includes sensory, nutritional, technological, convenience, and safety attributes (Kyriacou & Rouphael, 2018).

Cold storage reduces many reaction rates but can cause chilling injury and may alter volatile production. CA, MAP, and 1-MCP can retain firmness yet suppress characteristic aroma or delay ripening. Drying concentrates solids per unit mass but exposes heat- and oxygen-sensitive compounds; pretreatments may reduce browning yet create effluent or additive-labeling issues. Coatings can reduce water loss but create gloss, tack, altered gas transfer, or off-flavor. Ozone can reduce surface microorganisms without reaching protected sites, and excessive oxidation can damage tissue. These are reasons to use multi-endpoint studies and a retail-simulation period after storage.

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Food safety is not guaranteed by visual acceptability. Reduced-oxygen packaging can suppress aerobic spoilage cues while allowing tolerant hazards if temperature, pH, and product ecology are unfavorable. Hermetic storage controls insects but cannot detoxify contaminated grain. Re-circulated hydro-cooling water or wash water can spread contamination if disinfectant concentration, organic load, contact time, and replenishment are not controlled. Every technology should therefore sit within good hygienic practice, traceability, validated critical limits, and destination-market regulation.

7. Sustainability, Economics, and Adoption

Loss reduction is not automatically environmentally beneficial. The avoided product footprint should be compared with the intervention's capital equipment, electricity or fuel, refrigerant leakage, water, consumables, packaging, transport changes, and end of life. Refrigeration can prevent high-value perishable loss but also increases energy demand and refrigerant risk. Sustainable cold-chain planning combines right-sized equipment, efficient insulation and airflow, low-global-warming-potential refrigerants, leak control, renewable energy where reliable, heat recovery where useful, and high utilization (FAO & UNEP, 2022; James & James, 2010). Solar power can improve access but requires storage, backup, or operational scheduling to manage variability.

Techno-economic assessment should report capital expenditure, financing, operating energy and water, maintenance, replacement parts, consumables, labor, training, throughput, downtime, product value recovered, and sensitivity to seasonal utilization. A short laboratory shelf-life gain is not a payback calculation. For hermetic bags, reusable life, price seasonality, credit needs, and counterfeit risk may dominate. For CA rooms, packhouse scale, gas-tightness, backup power, and occupancy determine economics. For coatings or ozone, formulation or equipment service and regulatory verification may exceed the cost of the active ingredient.

Adoption is also institutional. Cooperative pre-cooling or drying hubs can spread capital across producers, but governance, scheduling, grading rules, and benefit sharing must be credible. Training should account for literacy and language, and technology design should consider who performs sorting, drying, loading, record keeping, and market sales. Labor displacement, worker exposure, gendered access to finance, and maintenance capability are part of sustainability, not

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external details. Repairability, local spare parts, and service response can be more important than peak efficiency.

Life-cycle assessment (LCA) and techno-economic analysis (TEA) are still uncommon in efficacy studies. Until comparative LCA/TEA data exist, claims such as “eco-friendly,” “low cost,” or “sustainable” should be identified as hypotheses or context-specific inferences. The appropriate comparison is the current local system, including its losses—not an idealized alternative.

8. Research Priorities

Five gaps constrain stronger recommendations. First, studies should adopt the minimum reporting set in Section 3.3 and define shelf-life failure before analysis. Second, independent multi-season and multi-location validation is needed for coatings, active films, ozone, and nano-enabled materials; laboratory pathogen inhibition does not establish commercial effectiveness. Third, postharvest trials should integrate microbiological safety, sensory acceptance, nutrition, and market grade rather than a single instrumental measure. Fourth, full-chain pilots should report temperature or moisture histories, equipment reliability, maintenance, and lot-level economic outcomes. Fifth, LCA and TEA should be paired with efficacy trials, including packaging disposal, refrigerant leakage, water treatment, and rebound effects.

For resource-constrained supply chains, research should compare modular and shared-service models: community drying with verified endpoints, aggregation-based pre-cooling, pay-per-use cold rooms, smaller hermetic units for partial withdrawal, and sensor systems whose alerts trigger feasible action. Readiness labels should distinguish laboratory proof of concept, pilot validation, initial commercial use, and widespread adoption. This prevents a novel material from being compared as if it had the same evidence and service ecosystem as refrigeration or hermetic storage.

9. Conclusion

Postharvest loss reduction is a system-design problem. The evidence does not support a universal 30-50% loss estimate, a generic 0-10 °C storage recommendation, fixed CA/MAP gas ranges, a single safe moisture or a_w threshold, or a two- to four-fold shelf-life extension across

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commodities. Each claim must be bounded by commodity, treatment, comparator, environment, duration, and endpoint.

For grains and pulses, validated drying, moisture-barrier packaging, sanitation, and hermetic storage provide accessible controls, but hermetic systems depend on dry grain, intact seals, and limited re-opening and do not remove existing mycotoxins. For fresh produce, shade, gentle handling, rapid commodity-compatible pre-cooling, and an unbroken cold chain remain the foundation. CA, MAP, 1-MCP, coatings, and ozone can add value when physiology, safety, sensory quality, authorization, and process control are validated. Nano-enabled packaging and digital twins merit targeted development, but migration, environmental fate, model validation, and regulatory readiness preclude broad claims.

The preferred intervention is therefore the simplest validated combination that controls the dominant loss mechanism, preserves safety and market quality, and can be financed, operated, maintained, and monitored in the intended chain. Comparative field evidence, LCA, TEA, and transparent readiness reporting are now more valuable than additional unbounded shelf-life claims.

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Bacteriocins in Postbiotic Preparations: Antimicrobial Potential, Safety and Translational Challenges

Faizan Tanvir[1*], Asia Latif Mughal[1], Hijab Zahra[1], Eiman Masood Malik[1], Areeba Mustafa[1], Hira Azhar[1], Almiza Ejaz[1], Hafiz Muhammad Usman[2], Rimsha Shabbir[1]

[1] Institute of Food and Nutritional Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

[2] Superior University, Lahore, Pakistan

Corresponding Author: faizantanvir344@gmail.com

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Abstract

Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins with established value in food bio preservation and growing interest as precision antimicrobials. Their relationship to postbiotics, however, is frequently described incorrectly. Under the International Scientific Association for Probiotics and Prebiotics consensus definition, a postbiotic must contain inanimate microorganisms and/or their components and confer a health benefit; a purified bacteriocin or a cell-free fermentation product is not automatically a postbiotic. This structured narrative review therefore asks where bacteriocins occur across distinct preparation types, how strongly observed effects can be attributed to them, and what evidence supports food, veterinary, or human-health translation. Literature indexed in PubMed and publisher databases was searched through 22 July 2026, with primary studies, consensus statements, and official regulatory evaluations prioritized. Nisin–lipid II binding and mannose-phosphotransferase-system recognition illustrate mechanism-specific activity, while the outer membrane generally restricts direct activity against Gram-negative bacteria. Food applications provide the strongest translational precedent, but performance depends on matrix composition, pH, processing, dose, and hurdle design. Animal studies support microbiome modulation and tolerability for selected molecules; studies of complex cell-free products cannot assign benefits to bacteriocins without chemical quantification or depletion controls. Resistance, cytotoxicity, immunogenicity, pharmacokinetics, delivery, and microbiome disruption require molecule- and route-specific evaluation. Regulatory acceptance of nisin A for defined food uses must not be generalized to other bacteriocins or therapeutic routes. Standardized preparation identity and evidence-linked terminology are prerequisites for credible translation.

Keywords: Bacteriocins, Postbiotics, Antimicrobial Peptides, Food Biopreservation, Safety

1. Introduction

Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins produced by bacteria and archaea. Many lactic acid bacteria make bacteriocins that inhibit competitors occupying the same ecological niche; several also inhibit foodborne or clinically relevant organisms. This diversity has made bacteriocins attractive as food preservatives, microbiome modulators, veterinary interventions, and leads for antimicrobial development (Cotter et al., 2005, 2013; Sugrue et al., 2024). Their appeal is strengthened by sequence programmability, biodegradability, and mechanisms that can differ from those of conventional antibiotics. These features are promising, but they do not establish low toxicity, broad-spectrum activity, resistance-proof efficacy, or clinical usefulness by themselves.

A second literature has developed around postbiotics. The current International Scientific Association for Probiotics and Prebiotics (ISAPP) definition is “a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” (Salminen et al., 2021). This definition is preparation-based rather than metabolite-based. Deliberately inactivated cells may be delivered with metabolites produced during growth or released during inactivation, but purified metabolites are not postbiotics. A cell-free supernatant that excludes microbial cells and cell structures also does not meet the definition merely because it was produced by a probiotic organism. Consequently, “bacteriocin,” “bacteriocin-containing cell-free product,” “inactivated-cell preparation,” and “live bacteriocin-producing probiotic” are not interchangeable terms.

Conceptual imprecision creates a causal problem. When a complex fermentation product improves an outcome, the effect may reflect organic acids, residual nutrients, exopolysaccharides, cell fragments, multiple peptides, or their interactions. A bacteriocin-specific mechanism requires at least identification and quantification of the molecule and is strengthened by purification, activity-guided fractionation, neutralization or deletion of the structural gene, and add-back experiments. Without such controls, attributing a host or antimicrobial effect to a bacteriocin is a hypothesis rather than a demonstrated conclusion.

This review therefore asks three linked questions: (i) in which preparation types do bacteriocins evidence that bacteriocins cause the reported antimicrobial or host effects; and (iii) what safety, resistance, formulation, and regulatory barriers separate food use from veterinary or human therapeutic translation? The novelty is an evidence-linked framework that treats preparation identity and causal attribution as prerequisites, rather than grouping purified molecules, cell-free products, inactivated cells, and live producers under a single label.

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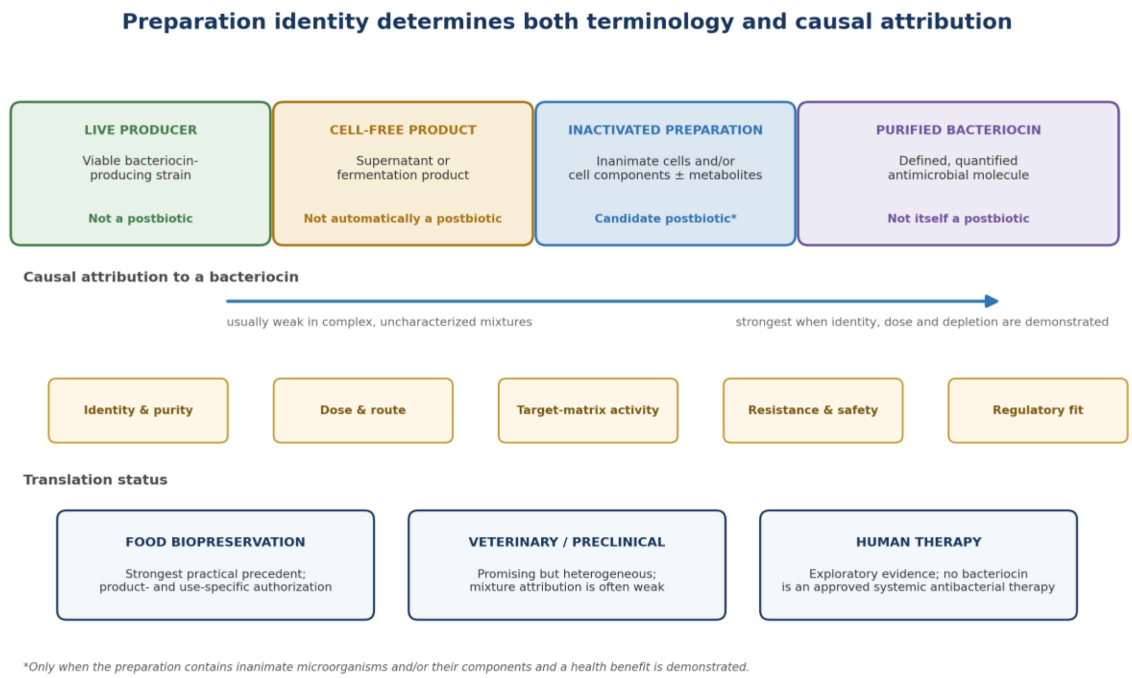

Figure 1. Evidence map linking preparation identity, bacteriocin attribution, development gates, and translation status. The figure was prepared for this review from the ISAPP definition and the evidence appraisal described in Section 2.

2. Literature Search and Evidence Appraisal

Searches combined bacteriocin*, nisin, pediocin, plantaricin, enterocin AS-48, postbiotic*, inactivated microorganism, cell-free supernatant, fermentation product, food preservation, food matrix, toxicity, haemolysis, immunogenicity, resistance, microbiome, pharmacokinetics, animal, clinical trial, and regulation. The principal date range was January 1989 to July 2026; earlier records were eligible when required to establish nomenclature or historical regulatory context. Reference lists of consensus papers, authoritative reviews, and official evaluations were searched backward, and bibliographic details were checked against DOI, PubMed, publisher, or regulatory records.

English-language peer-reviewed primary studies were prioritized when they identified the bacteriocin, described the preparation or inactivation procedure, and reported a relevant food, antimicrobial, safety, animal, or human endpoint. Consensus statements and official regulatory evaluations were included for definitions and legal status. Studies were excluded from bacteriocin-specific conclusions when they evaluated an uncharacterized probiotic, a generic postbiotic product, or a fermentation mixture without evidence that a bacteriocin was present. Reviews were used for scope and cross-checking but not as substitutes for primary toxicology where the original report was available. Evidence was appraised narratively because preparations, organisms, doses, matrices, routes, and endpoints were too heterogeneous for quantitative synthesis. Human controlled evidence was ranked above animal in vivo, ex vivo/in vitro, and mechanistic or indirect evidence. Causal attribution was rated strong when a defined molecule and dose were tested; moderate when a bacteriocin-producing strain or combination was tested with relevant controls; and weak when a complex mixture lacked bacteriocin quantification, depletion, or genetic controls.

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3. Conceptual Boundaries: When a Bacteriocin Is—and Is Not—Part of a Postbiotic

Table 1 separates the four preparation types most often conflated in this field. A bacteriocin may be present in all four, but the correct name, safety question, and strength of causal inference differ. The word "postbiotic" is appropriate only for an inactivated-microorganism preparation that contains the required microbial material and for which a health benefit has been demonstrated. Inactivation alone establishes neither benefit nor postbiotic status. Conversely, purification can establish chemical identity and strengthen causal attribution while moving the product outside the postbiotic definition (Salminen et al., 2021).

Table 1. Preparation categories, terminology, and bacteriocin attribution.

Preparation type	What is administered	Postbiotic status	Bacteriocin attribution	Preferred reporting
Purified bacteriocin	Chemically or chromatographically defined peptide/protein; viable cells absent.	Not a postbiotic; it is a microbial metabolite/component tested as a defined molecule.	Potentially strong if identity, purity, activity units or molar dose, and comparator are reported.	Molecule/variant, purity, mass, activity assay, dose, route, formulation, contaminants.
Bacteriocin-containing cell-free supernatant or fermentation product	Soluble fermentation products after centrifugation/filtration; cells and much cell structure removed.	Not automatically a postbiotic under the ISAPP definition.	Usually weak unless the bacteriocin is quantified and depleted, neutralized, or genetically removed.	Producer strain, culture conditions, filtration, pH neutralization, composition, bacteriocin identity/concentration.
Inactivated whole-cell preparation ± metabolites	Inanimate microorganisms and/or cell components, potentially with retained fermentation metabolites.	Candidate postbiotic only after a health benefit is demonstrated.	Mixture-level effect; bacteriocin-specific attribution requires additional controls.	Strain, biomass, inactivation and viability verification, retained components, dose, stability, benefit endpoint.
Live bacteriocin-producing probiotic or protective culture	Viable producer organism, sometimes with in situ bacteriocin production.	Not a postbiotic; may be a probiotic or protective culture if the relevant criteria are met.	Moderate only when bacteriocin-null and complemented strains or direct in situ measurements are used.	Strain identity, viability, colonization/persistence, production in target matrix, genetic controls, dose.

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Figure 1 translates this distinction into a development pathway. Preparation identity comes first; analytical, safety, matrix, and regulatory gates then determine whether evidence can support a food use, a veterinary/preclinical claim, or a human therapeutic claim. The framework deliberately separates regulatory authorization from efficacy: permission to use nisin A in particular foods does not demonstrate that a nisin formulation treats infection.

4. Classification, Biosynthesis, and Mechanisms

4.1 Classification and biosynthesis

Bacteriocin classification has changed as genome mining and structural chemistry have expanded the field. A practical, non-exclusive framework distinguishes post-translationally modified ribosomally synthesized and post-translationally modified peptides (RiPPs; commonly called class I), small largely unmodified peptides (class II), and larger bacteriolytic or non-lytic proteins (class III). Class I includes lanthipeptides such as nisin, whose dehydrated amino acids and lanthionine bridges arise from dedicated maturation enzymes. Class II includes pediocin-like one-peptide bacteriocins, two-peptide systems, leaderless peptides, and circular peptides, although the placement of circular and leaderless bacteriocins differs between schemes. Class III contains larger, often heat-labile proteins. Size cut-offs and subclass labels should therefore be cited to a defined scheme rather than presented as universal facts (Alvarez-Sieiro et al., 2016; Sugrue et al., 2024).

Biosynthetic loci commonly encode the precursor peptide, modification and export functions, self-immunity, and regulatory machinery. Their location on chromosomes, plasmids, or mobile elements affects stability and transfer. Production is also phenotype-dependent: strain genotype does not establish that an active concentration is reached in a food, intestine, or formulation. Reports should connect genomic prediction to peptide mass, sequence or structural confirmation, and quantitative activity under the intended conditions.

4.2 Mechanism, spectrum, and the Gram-negative barrier

Mechanisms are molecule-specific. Nisin binds the pyrophosphate region of lipid II, thereby sequestering an essential peptidoglycan precursor and organizing membrane pores at low local concentrations (Wiedemann et al., 2001). By contrast, many class IIa pediocin-like bacteriocins recognize the membrane IIC/IID components of a subset of mannose phosphotransferase systems; receptor sequence and expression help define susceptibility (Kjos et al., 2010). Other bacteriocins disrupt membranes without a single known receptor, inhibit enzymes, or degrade cell walls. It is therefore inaccurate to describe every bacteriocin as a nonspecific pore former.

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Spectrum is equally contextual. Many lactic-acid-bacterial bacteriocins are most active against Gram-positive organisms because their cytoplasmic membrane or cognate receptor is accessible. The outer membrane of Gram-negative bacteria impedes many peptides. Activity against Escherichia coli or Salmonella often appears only after membrane permeabilization, high pressure, heating, acidification, chelation, or combination with another antimicrobial. For example, nisin at 10 μM combined with trans-cinnamaldehyde and EDTA delayed growth of porcine E. coli strains in vitro, but the result belongs to the combination and cannot be assigned to nisin alone (Field et al., 2017). Statements of "broad-spectrum" activity must therefore specify strain panel, growth phase, matrix, pH, assay, exposure, and whether a hurdle agent was required.

Potency values are not automatically comparable. Agar diffusion depends on peptide diffusion and indicator physiology; activity units depend on the assay definition; and MICs vary with inoculum, medium, adsorption, and purity. Molar concentration of an identified molecule is preferable for mechanistic comparisons, while food challenge studies should additionally report activity per gram or milliliter, recovery over time, and survivor counts.

5. Food-System Applications and Matrix Performance

5.1 Modes of use

Food biopreservation provides the clearest practical precedent for bacteriocins. Three strategies are distinct: direct addition of a purified or standardized preparation; incorporation into a coating, film, or packaging system; and in situ production by a starter or protective culture (Deegan et al., 2006). Direct addition offers a known input but not necessarily a known active dose after processing. A producer culture may generate activity gradually and locally, yet its performance depends on growth, competition, phage sensitivity, and regulation of the biosynthetic locus. In Cheddar manufacture, a Lactococcus lactis strain producing lacticin 3147 demonstrated the feasibility of integrating a bacteriocinogenic starter into a real fermentation process (Ryan et al., 1996). That is evidence for a live culture strategy, not for a postbiotic. Nisin is particularly useful against susceptible Gram-positive vegetative cells and can inhibit spore outgrowth in some formulations. Nevertheless, attached bacteria and complex surfaces reduce. Nisin is particularly useful against susceptible Gram-positive vegetative cells and can inhibit spore outgrowth in some formulations.

Nevertheless, attached bacteria and complex surfaces reduce predictability; early work on meat showed that nisin delayed growth of susceptible attached bacteria but was insufficient as a stand-alone control across all organisms tested (Chung et al., 1989). Food-safety conclusions should be based on target-pathogen challenge tests in the intended product, not on broth inhibition zones.

5.2 Matrix, processing, and hurdle effects

Food composition can help or hinder activity. Low pH often improves nisin solubility and stability, whereas neutral or alkaline conditions can reduce recoverable activity. Proteins, fat droplets, phospholipids, and suspended particles can bind bacteriocins or divert them from bacterial targets. Salt, water activity, indigenous microbiota, bacterial stress state, and proteases further alter efficacy. Thermal processing may preserve many small class I and II peptides while changing the target cell envelope; large class III proteins are generally more heat-sensitive. These interactions explain why a concentration effective in laboratory broth may fail in cheese, meat, or an emulsion.

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Rational hurdles can lower the required bacteriocin dose and broaden coverage. Acidification, mild heat, high pressure, chelators, essential-oil components, or cell-envelope-active antimicrobials may sensitize targets. Hurdles must be evaluated for additive toxicity, sensory acceptability, labelling, and legal status, and synergy should be demonstrated quantitatively rather than inferred from a larger inhibition zone. The nisin–cinnamaldehyde–EDTA example illustrates both the opportunity and the attribution constraint (Field et al., 2017).

Commercial feasibility requires more than killing a challenge organism. Production titre, downstream recovery, purity specification, batch potency, residual culture media, stability during storage, compatibility with the process, flavour or texture effects, and cost per treated unit all matter. A food-grade producer does not make every secreted molecule food-approved, and the safety of the source organism cannot substitute for characterization of the final preparation.

6. Evidence Beyond Food Preservation

6.1 Defined bacteriocins and microbiome modulation

A defined bacteriocin can reshape a microbial community as well as suppress a pathogen. O'Reilly et al. (2023) administered nisin powder or encapsulated nisin to 40 male piglets (10 per group) for four days. Intact, bioactive nisin reached the lower gastrointestinal tract, and treatment produced reversible changes in community composition and short-chain-fatty-acid profiles. This study provides direct evidence that orally delivered nisin can remain active in vivo, but it also contradicts the assumption that bacteriocins inherently spare commensal microbiota. Whether a change is beneficial depends on indication, duration, baseline ecology, dose, and recovery.

Local human administration has been explored on a small scale. In a four-day plaque-regrowth study, 45 participants were randomized to nisin Z extract, distilled water, or 0.2% chlorhexidine mouthrinse (15 per group). Nisin showed antiplaque activity but was less effective than chlorhexidine (Mitra et al., 2019). The short duration, local route, small sample, and incompletely standardized extract prevent extrapolation to systemic infection or long-term oral health. It is best treated as exploratory human evidence rather than clinical validation of bacteriocins as antibiotic replacements.

6.2 Complex products and veterinary evidence

Complex fermentation products may benefit animal performance or intestinal endpoints, but terminology and attribution require care. Chang et al. (2022) fed 245 broiler chickens one of three Lactiplantibacillus plantarum cell-free fermentation products, a basal diet, or oxytetracycline. The products were produced by centrifugation and 0.22-µm filtration and contained measurable organic acids and antioxidant activity. Improvements were reported in selected growth, barrier, immunological, and microbial outcomes. Because cells were removed, these preparations do not meet the current ISAPP postbiotic definition on the reported processing alone. Moreover, the animal study did not identify a bacteriocin dose or use depletion or gene-knockout controls. The results support the activity of the fermentation products as mixtures, not a bacteriocin-specific mechanism.

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The same caution applies to inflammatory bowel disease, sepsis, obesity, or metabolic claims attributed to generic postbiotics. Evidence that an inactivated preparation or fermentation product modifies inflammation does not establish that its bacteriocin component caused the effect. A defensible chain of evidence requires the molecule to be detected at the administered dose, remain stable or reach the target site, reproduce the effect when purified, lose the effect when selectively removed, and retain an acceptable safety window.

6.3 Representative evidence and attribution

Table 2 compares representative studies using the preparation and attribution framework. It is an evidence map rather than an exhaustive efficacy table. The most informative contrast is between a defined bacteriocin experiment and a complex product for which bacteriocin identity and concentration were not reported.

Table 2. Representative bacteriocin-related evidence, preparation identity, and strength of attribution.

Study / setting	Organism or source	Preparation / inactivation	Bacteriocin identity and dose	Model / sample	Comparator	Main outcome
Wiedemann et al. (2001); mechanism	Nisin from Lactococcus lactis	Purified peptide; no cells	Nisin; defined biochemical concentrations	Model membranes and biochemical assays	Membranes with/without lipid II	Lipid II binding combined pore formation with inhibition of cell-wall synthesis.
Ryan et al. (1996); Cheddar cheese	Bacteriocin-producing L. lactis starter	Live producer culture; not inactivated	Lacticin 3147 produced in situ; recovered activity rather than purified dose	Pilot cheese manufacture	Conventional starter/control manufacture	Demonstrated feasibility of a bacteriocinogenic starter in cheese processing.
Field et al. (2017); antimicrobial combination	E. coli strains of swine origin	Purified/standardized nisin in a combination	Nisin 10 μM + trans-cinnamaldehyde 125 μg/mL + EDTA 0.25–2%	In vitro growth assays	Single components and untreated controls	Combination extended growth inhibition, including enterotoxigenic strains.

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O'Reilly et al. (2023); gut microbiome	Nisin; porcine intestinal microbiota	Nisin powder or encapsulated nisin; no cells	150 mg/kg body weight; oral/feed exposure for 4 days	40 male piglets; 10/group	Untreated and encapsulant controls	Active nisin reached faeces; microbiota and SCFA changes were largely reversible after withdrawal.
Chang et al. (2022); broiler gut health	L. plantarum RG11, RI11, RS5	Centrifuged and 0.22-µm-filtered cell-free fermentation products	Bacteriocin identity/concentration not quantified in the feeding study; 0.1% v/w product	245 male Cobb 500 broilers; 5 groups, 7 replicates × 7 birds; 42 days	Basal diet and 0.01% oxytetracycline	Selected growth, barrier, immune and microbial outcomes improved.

7. Resistance and Safety

7.1 Resistance is possible and mechanism-dependent

Bacteriocins should not be described as resistance-proof. Stable or adaptive tolerance can arise through receptor loss or altered expression, cell-envelope charge and fluidity changes, stress responses, proteolysis, efflux, biofilm state, and dedicated immunity determinants. For pediocin-like bacteriocins, reduced or altered mannose phosphotransferase-system function can markedly change susceptibility (Kjos et al., 2011). In Listeria monocytogenes, cell-wall changes associated with pbp2229 expression increased nisin resistance and cross-protection to class IIa bacteriocins (Gravesen et al., 2004). The fitness and virulence effects of resistance may be favourable or unfavourable and must be measured rather than assumed.

Food and clinical development should therefore include spontaneous resistance frequency, serial passage, cross-resistance to other bacteriocins and antibiotics, stability after withdrawal, performance in biofilms, and genomic or transcriptomic characterization. Multi-hurdle design can suppress escape, but sublethal gradients in foods or delivery systems can also select tolerant populations. Surveillance is relevant even for products regarded as natural.

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7.2 Toxicology, host selectivity, pharmacokinetics, and delivery

Safety is molecule-, formulation-, dose-, route-, and population-specific. A peptide tolerated after ingestion may behave differently when inhaled, applied to damaged mucosa, or injected. Food exposure does not establish systemic safety. Development programs should test identity and impurities, cytotoxicity across relevant human cells, haemolysis, local irritation, sensitization and immunogenicity, genotoxicity where indicated, repeated-dose organ toxicity, reproductive or developmental endpoints when exposure warrants them, and effects on the resident microbiome (Soltani et al., 2021). For therapeutic use, absorption, proteolysis, tissue distribution, clearance, anti-drug antibodies, and delivery-site concentrations are central.

Table 3. Representative primary toxicology studies of bacteriocins or bacteriocin preparations.

Bacteriocin / preparation	Study design and sample	Exposure	Reported findings
Nisin A commercial preparation Hagiwara et al. (2010)	F344 rats; 10 males and 10 females/group; 90-day dietary study; NaCl reference group	0, 0.2, 1.0, or 5.0% commercial preparation; highest intake ≈2,996 mg/kg/day males and 3,187 mg/kg/day females; ≈224.7 and 239 mg active nisin A/kg/day	No toxicologically significant treatment-related changes; highest dose was the study NOAEL.
Enterocin AS-48 Baños et al. (2019)	BALB/c mice; 90-day dietary study; nisin comparator	50, 100, or 200 mg/kg diet	No deaths or major clinical/biochemical toxicity; focal hepatocyte vacuolar degeneration occurred in some animals at 100–200 mg/kg.
Enterocin AS-48 Cebrián et al. (2019)	Human-cell and blood assays; mouse skin sensitization; zebrafish embryos	In vitro concentrations up to 27 µM; topical sensitization	Low haemolysis/cytotoxicity at proposed antimicrobial concentrations and

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		doses 1, 10, 20 µg/5 µL	little pro-inflammatory activity; zebrafish embryos were more sensitive.
P34 preparation (Vaucher et al., 2011)	BALB/c mice; acute oral and 21-day repeated exposure; nisin comparator	Acute 82.5–330 mg/kg; repeated 0.825 mg/kg/day	No mortality; estimated LD50 above the highest tested dose; limited serum changes, with histological findings reported.
TSU4 Sahoo et al. (2017)	Male BALB/c mice; immunogenicity, acute oral and 21-day repeated exposure	Acute 50, 100, 200 mg/kg; repeated 0.5 mg/kg/day	No mortality and no major serum-biochemical or histological abnormalities; LD50 reported above 200 mg/kg.
Pediocin N6 powder Ketaren et al. (2016)	Male Swiss mice; n=5/dose; single-dose study with 15-day observation	5,000, 10,000, 15,000, or 20,000 mg powder/kg	No deaths; LD50 could not be calculated and was inferred to exceed 20,000 mg powder/kg.

Table 3 reconstructs representative primary toxicology evidence. Acute LD50 limits, repeated-dose NOAELs, and absence of observed mortality answer different questions and are not interchangeable. Nominal doses of commercial preparations also must not be reported as active-peptide doses. For example, Hagiwara et al. (2010) tested a commercial nisin preparation containing approximately 7.5% active nisin A; the highest preparation intake was about 3,000 mg/kg body weight/day, corresponding to approximately 225–239 mg active nisin A/kg body weight/day.That repeated-dose NOAEL is not an LD50 and does not define safety for parenteral nisin.

8. Regulatory Status and Commercial Translation

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Regulation follows the product, jurisdiction, intended use, dose, and route—not the general word bacteriocin. In the United States, 21 CFR §184.1538 addresses a defined nisin preparation for specified food uses and manufacturing conditions (U.S. Food and Drug Administration, 2026). This is a food-ingredient provision, not approval of nisin as a drug or of every bacteriocin-producing organism. The European Food Safety Authority likewise evaluated nisin (E 234) for food-additive exposure rather than therapeutic use (EFSA Panel on Food Additives and Nutrient Sources Added to Food, 2017). The Joint FAO/WHO Expert Committee on Food Additives evaluation applies specifically to nisin A and reports an acceptable daily intake of 0–2 mg/kg body weight expressed as nisin A (JECFA, 2024). That value should not be transferred to pediocin, plantaricin, AS-48, a crude supernatant, or a parenteral product.

Regulatory submissions for a purified molecule generally require structural identity, purity and impurity profiles, validated potency, manufacturing controls, stability, exposure, and indication-specific safety and efficacy. A complex inactivated-cell preparation requires strain-level identity, absence of viable cells, inactivation validation, composition, batch consistency, and evidence for the claimed health benefit. A live producer introduces additional questions of viability, genetic stability, antimicrobial-resistance genes, environmental release, and in situ production. These paths may fall under food additive, feed additive, novel food, dietary supplement, veterinary medicinal product, drug, or biologic frameworks depending on jurisdiction and claim.

Commercial food use of nisin therefore demonstrates that a bacteriocin can be manufactured, standardized, and regulated for a circumscribed purpose. It does not validate “bacteriocins as therapeutic proteins” as a class. As of the search date, the peer-reviewed landscape remains dominated by in vitro and animal studies, and no bacteriocin discussed here is established as an approved systemic antibacterial therapy for humans (Sugrue et al., 2024).

9. Production, Standardization, and Research Priorities

Translation is most likely when discovery and product design are linked from the outset. High titre alone is insufficient if a peptide is unstable, adsorbs to biomass, is costly to purify, or loses activity in the target matrix. Fermentation optimization should report producer strain, medium, pH, temperature, aeration, induction, growth phase, volumetric activity, peptide concentration, and recovery yield. Downstream processing should distinguish crude activity units from mass of active peptide and document residual solvents, media components, endotoxin where relevant, and congeners.

For inactivated-cell preparations, standardization must extend beyond the bacteriocin. Thermal, pressure, irradiation, or chemical inactivation can change cell-wall architecture, release intracellular material, degrade peptides, and alter immunological activity. Viability should be assessed with methods capable of detecting injured survivors, and the retained biomass and metabolite profile should be quantified. Batch release criteria should connect chemical identity to a validated biological assay.

Six priorities would make the field more reproducible and clinically credible:

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- Use evidence-linked terminology: state whether the test article is a purified molecule, cell-free product, inactivated-cell preparation, or live producer, and reserve "postbiotic" for preparations meeting the consensus definition and benefit criterion.
- Establish causality in mixtures using targeted quantification, activity-guided fractionation, immunoneutralization, bacteriocin-null mutants, selective depletion, and add-back controls.
- Report concentration in interpretable units, including molar or mass dose of active peptide, purity, activity-unit definition, formulation, route, and recovered activity at the target site.
- Use target-matrix and target-population models: food challenge tests, polymicrobial biofilms, relevant animal disease models, and adequately powered human trials with pre-specified endpoints.
- Build resistance and microbiome endpoints into efficacy studies rather than treating them as afterthoughts; include recovery after exposure and cross-resistance testing.
- Develop route-specific safety and pharmacokinetic packages and avoid extrapolating food authorization or acute oral tolerability to chronic or invasive therapeutic use.

A minimum evidence package for a bacteriocin-containing postbiotic should therefore include: confirmed strain identity; validated inactivation and absence of viable cells; quantitative biomass and composition; bacteriocin identity, concentration and stability; a demonstrated health benefit of the complete preparation; and bacteriocin-specific controls if the molecule is claimed as the active principle. This package would allow reviewers and regulators to distinguish a credible mechanism from attractive but untested labelling.

Conclusion

Bacteriocins are a diverse antimicrobial class with a strong food-biopreservation precedent and important mechanistic and preclinical potential. Their value is diminished, not enhanced, when purified bacteriocins, cell-free fermentation products, inactivated-cell preparations, and live producer strains are collapsed into the single term postbiotic. Under the current consensus definition, a purified bacteriocin is not itself a postbiotic, and a cell-free product is not automatically one.

The strongest evidence supports defined mechanisms, selected food applications, and molecule-specific oral or local preclinical studies. Human therapeutic evidence remains limited, and complex-product studies usually cannot assign host benefits to bacteriocins without analytical and causal controls. Resistance can emerge; microbiomes can be altered; and safety depends on the molecule, formulation, dose, route, duration, and population. Nisin A’s food regulatory status is an important precedent but cannot be generalized to other bacteriocins or therapeutic indications.

Future progress depends on precise preparation identity, quantitative reporting, target-matrix validation, resistance surveillance, route-specific toxicology, and controlled clinical evaluation. With those standards, bacteriocins may become useful components of postbiotic preparations or independent antimicrobial products—two related but scientifically and regulatorily distinct development paths.

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Fermented Mushroom-Chickpea Protein Nuggets: Development and Evaluation With Uncycled Binders For Sustainable Nutrition

Hamna Nisar[1*], Humayun Ajaz[1*]

[1*] Department of Food Engineering and Biotechnology, University of Engineering and Technology, Lahore, Pakistan

Corresponding Author: hamnanisar110@gmail.com

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Abstract

In this study, a unique vegetal protein product fermented mushroom chickpea nuggets is produced and tested as a response to Pakistan's rising nutritional demand and global environmental challenges. By taking advantage of locally grown mushrooms (*Pleurotus ostreatus, Agaricus bisporus*) and chickpeas (*Cicer arietinum L.),* the formulation was added with fermentation and addition of some selected probiotic strains (*Lactobacillus plantarum 299v, L. delbrueckii, L. helveticus and Streptococcus thermophilus*) to enhance the product. Aquafaba was a byproduct reuse ingredient of cooking chickpeas which worked as a binding agent that was sustainable and functional. The objective of the research was to (i) formulate fermented nuggets; (ii) measure pH, proximate composition, and microbial count; and (iii) determine sensory acceptability in terms of use of trained panelists. Added value through the fermentation process was increased nutritional values of the fermented formulations; protein levels of (26.8%,) moderate amount of fat (8 9%) and up to 7% of fibers were retained. The pH levels (4.21-4.66) showed ideal maturation conditions and microbial cell counts amply demonstrated the proliferation of lactic acid bacteria that was below the safe food levels. Sensory evaluation by trained panelist identified 50:50 mushroom to chickpea formulations the most preferred due to its flavor, texture and acceptability. These findings showed that the feasibility of fermentation technology in the production of functional-improved nutrition, safety and acceptability of plant-based products. These developments would provide a future commercialization channel, especially in the frozen food industry and institutionalized food programs in Pakistan, as part of the national food security and sustainability objectives.

Keywords: Mushroom, Chickpea, Plant based Protein, Nuggets, Sustainable Nutrition

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Introduction

The global food system is under unprecedented pressure due to rapid demographic and socioeconomic transformations. According to the Food and Agriculture Organization of the United Nations (FAO, 2017), the world's population is projected to reach approximately 9.7 billion people by 2050, an increase of nearly 2 billion people from today. This rapid growth, especially concentrated in developing regions of Asia and Africa, is driving a steep rise in demand for proteins. Globally, food production will need to increase by an estimated 60% to 70% to meet the nutritional requirements of this burgeoning population (FAO, 2009). Protein is one of the most vital macronutrients necessary for proper growth, muscle repair, enzymatic function, and immune defense.

The Pakistan National Nutrition Survey 2018 (Government of Pakistan & UNICEF, 2019) shows that over 58% of Pakistani households face some level of food insecurity, and that protein-energy malnutrition is prevalent across all provinces especially among children under five and women of reproductive age (Gillani et al., 2022). More alarmingly, stunting affects over 40% of children under five in Pakistan, with protein deficiency cited as a key contributor. Similarly, diets in Pakistan lean heavily on cereals (mostly wheat and rice), providing mostly carbohydrates, with protein consumption often relying on pulses or expensive and environmentally costly animal sources like meat and dairy.

Pakistan's existing protein sources can be broadly divided into two categories: animal-based and plant-based. Poultry, beef, goat, and dairy products remain important protein sources in Pakistan. However, these products come with a substantial environmental footprint livestock farming accounts for a significant proportion of greenhouse gas emissions (GHGs), water consumption, and land use. Animal-protein production also places substantial demands on land and water and contributes to greenhouse gas emissions (Nolden & Forde, 2023). Rapidly rising feed prices and energy costs have further pushed up meat and dairy prices by over 35% since 2020 (Pakistan Bureau of Statistics, 2023), making them less affordable for average households.

Although soy protein is one of the most commonly used plant-based proteins worldwide due to its high protein content (~36–40% dry basis), soy also presents serious drawbacks especially in terms of allergenicity and environmental impact. Soybean is among the major allergenic foods subject to mandatory labeling in many jurisdictions (Ham et al., 2025). Pakistan, though not a significant soy producer, imports over 2.8 million tons of soybean annually (Ministry of National Food Security and Research, 2022) contributing indirectly to international environmental concerns while spending substantial foreign exchange on soy imports.

Thus, exploring diverse local crops (e.g. chickpeas) and novel protein sources (e.g. edible mushrooms) as protein ingredients is more sustainable for Pakistan. Mushrooms and chickpeas require fewer agricultural inputs, thrive under local conditions, and produce low-carbon, nutrient-dense food. Combining them with fermentation can further enhance protein bioavailability and taste an important factor for ensuring consumer acceptance of these sustainable proteins (Rajpurohit & Li, 2023; Webb et al., 2023).

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Mushrooms and pulses like chickpeas are particular example of the perfect protein synergy against Pakistan's ever increasing protein requirement. Mushrooms (oyster, button etc.) are a rich source of bioavailable proteins (20–30% dry weight), B vitamins, ergosterol (a precursor of vitamin D2) and antioxidant compounds such as ergothioneine and glutathione. Mushrooms are also inherently low in fat and cholesterol, making them suitable for health-conscious consumers. Legumes like chickpeas (Cicer arietinum) are an agricultural staple across Pakistan and India, containing 17–22% protein, 18–22 g/100 g dietary fiber, and substantial minerals like iron, zinc, and calcium. When combined, the complementary amino acid profiles of mushrooms and legumes produce a protein profile comparable to or even superior to some animal-derived proteins. Such synergy can combat the widespread protein-energy malnutrition of Pakistan (Boukid, 2021; Grasso et al., 2022; Wang & Zhao, 2023).

Fermentation is an age-old method of food processing that is enjoying a Renaissance as part of the movement of sustainable food innovation. At its simplest level, fermentation harnesses the power of beneficial microorganisms primarily lactic acid bacteria or LAB to take raw ingredients and turn them into foods that are safer (lacking the bad kind of bacteria), more nutritious (rich in probiotics and other beneficial nutrients) and more flavorful. Fermentation is an important strategy for enhancing plant protein products such as mushroom-chickpea nuggets as it improves protein digestibility, increases bioavailability of essential amino acids and also reduces anti-nutritional factors such as phytic acid and tannins. Fermentation, by lowering the pH of the food matrix, has natural preservative effect that inhibits the growth of pathogenic microorganisms– which is congruent with the food safety of packaged food adopted by the Pakistan (Sharma et al., 2020).

Concurrently, the use of upcycled binders further supports sustainability and resilience in food systems. Pakistan's agricultural sector generates food-processing by-products such as okara (soy pulp), aquafaba (chickpea cooking water), spent grains, and vegetable trimmings. Substantial postharvest losses create opportunities to recover these nutrient-rich materials as functional ingredients (Pakistan Agricultural Research Council, 2023). By incorporating upcycled binders for example, aquafaba (the viscous water left after cooking chickpeas) into the product formulation, food developers can replace costly commercial binders like egg white powder and gums, lowering production costs and making the product accessible at scale. Aquafaba, rich in polysaccharides and proteins, mimics the functional properties of egg white's emulsification, foaming, and gel formation resulting in a smooth, stable nugget matrix (Ali et al., 2025; Augustin et al., 2024).

Plant-based protein product development has evolved significantly over the last decade, with a strong emphasis on combining mycoproteins (from mushrooms) and legumes to create sustainable, nutritious alternatives to animal-based foods. Several global studies have demonstrated the effectiveness of using fermentation to improve the nutritional, textural, and sensory qualities of such products (Cocan et al., 2022; Mazumder et al., 2023; Moorthi et al., 2022; Sharma et al., 2020; Wang & Zhao, 2023; Yusof et al., 2021). However, despite the promise shown internationally, research and industrial application of fermented mushroom-legume products remain underdeveloped in Pakistan and much of South Asia.

Despite these promising solutions, there remains a significant knowledge gap in Pakistan's food research. Specifically: Data on mushroom-legume fermentation in nugget production is sparse most literature focuses on soy, wheat, or cereal-based analogues. No comprehensive research

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exists on using upcycled binders in mushroom-chickpea protein formulations for improving texture and nutritional profiles. The sensory acceptability and microbiological stability of such fermented plant-based nuggets tailored to Pakistani consumers have yet to be systematically investigated. This research, therefore, aims to bridge this gap by developing a fermented mushroom-chickpea protein nugget with upcycled binders that is not only nutritionally sound and microbiologically safe but also economically viable and well-accepted by Pakistani consumers.

Despite growing global interest in plant-based and fermented protein products, there remains a significant research gap in Pakistan regarding the development of nutritionally enhanced, microbiologically safe, and sensory-acceptable alternatives to conventional animal-based protein foods. Existing literature largely focuses on soy-based, cereal-based, or non-fermented plant protein products, while limited attention has been given to mushroom–legume combinations, particularly in structured, ready-to-cook combination such as nuggets. Moreover, fermentation practices in Pakistan are predominantly traditional in nature, with minimal application of controlled starter cultures for improving the nutritional quality, safety, and sensory characteristics of plant-based foods.

Additionally, although food processing by-products such as aquafaba are generated in substantial quantities, their utilization as functional, upcycled ingredients in value-added food product development remains largely unexplored in the local context. There is also a lack of comprehensive local data evaluating the combined effects of fermentation on proximate composition, physicochemical parameters, microbial safety, and consumer acceptability of mushroom–chickpea based products developed using sustainable and locally available resources.

The novelty of the present study lies in the integrated development of fermented mushroom–chickpea protein nuggets using defined lactic acid bacteria starter cultures and an upcycled binder (aquafaba) within a single formulation framework. This research provides a systematic evaluation of fermentation-induced changes in nutritional composition, pH, microbiological quality, and sensory acceptability, thereby generating locally relevant scientific data. To the best of the author's knowledge, this is among the first studies in Pakistan to combine edible mushrooms, chickpeas, controlled fermentation, and upcycled binders in a nugget-type product aimed at addressing protein malnutrition, sustainability, and consumer acceptability simultaneously. The primary objective of this research was to develop and evaluate fermented mushroom–chickpea protein nuggets incorporating upcycled binders as a sustainable, nutritionally enhanced, and sensory-acceptable alternative to conventional protein-based snacks, specifically tailored to Pakistan's food consumption patterns and resource limitations.

1. To develop fermented protein-rich nuggets with a base of oyster and button mushrooms, chickpeas, and upcycled binders (e.g. aquafaba), utilizing fermentation techniques for product enhancement.
2. To quantify and compare changes in proximate composition (protein, fat, fiber, moisture, and ash), pH, and microbiological safety (TPC) after a controlled fermentation process (48 hours) versus a non-fermented control.
3. To Assess the sensory acceptability (appearance, flavor, texture, aroma, and overall preference) of fermented mushroom–chickpea nuggets relative to non-fermented counterparts to evaluate potential market acceptance.

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Materials And Methods

2.1 Overview of fermentation in food development

The fermentation process is central to this study as it aims to enhance nutritional bioavailability, improve microbial safety, and enrich sensory qualities of the final nugget product. Fermentation is a metabolic process where microorganisms, particularly lactic acid bacteria (LAB), convert carbohydrates into organic acids. In plant-based food systems, it improves protein digestibility, flavor depth and aroma development, reduction in anti-nutritional factors (e.g., phytates, tannins), and microbial stability through natural acidification. This makes fermentation highly suitable for transforming chickpeas and mushrooms into functional, shelf-stable food products.

2.2 Raw materials

Fresh oyster mushrooms (*Pleurotus ostreatus*), button mushrooms (*Agaricus bisporus*), and chickpeas (*Cicer arietinum* L.) were used as the primary raw materials. Aquafaba, the cooking water obtained from chickpeas, was used as an upcycled binding agent. All raw materials were procured from the local market and transported to the laboratory under hygienic conditions.

2.3 Substrate preparation

Mushroom pre-processing

Fresh oyster and button mushrooms were washed thoroughly with distilled water to remove visible soil and debris. Mushrooms were soaked in 0.1% potassium metabisulfite (KMS) solution for 2 minutes to reduce microbial load and then rinsed twice with sterile distilled water to remove chemical residue. The mushrooms were sliced into uniform 5 mm pieces using sterilized knives to increase surface area for fermentation. Processed mushrooms were stored in airtight containers at 4°C and used within 12 hours.

Chickpea pre-processing

Chickpeas were soaked in distilled water in a 1:3 (w/v) ratio for 12–16 hours at room temperature (25 ± 2°C). The soaked chickpeas were autoclaved at 121°C under 15 psi pressure for 30 minutes to eliminate microbial contaminants, deactivate anti-nutritional factors, and soften texture. After autoclaving, chickpeas were allowed to cool to 25°C under sanitary conditions prior to fermentation.

2.4 Probiotic strains and inoculum preparation

The lactic acid bacteria strains used in this study included *Lactobacillus plantarum* 299v, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*. The strains were activated in de Man, Rogosa and Sharpe (MRS) broth and incubated anaerobically at 37°C for 24 hours. Cultures were adjusted to an optical density (OD_{600})

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of 1.0, corresponding to approximately 10^8 CFU/mL. Cultures were stored at 4°C and used within 48 hours.

2.5 Experimental design and fermentation batches

Three fermentation batches were prepared based on substrate composition and starter culture combinations:

T1: Mushroom + Chickpea + Aquafaba inoculated with *L. plantarum*, *L. delbrueckii*, and *S. thermophilus*

T2: Mushroom + Chickpea inoculated with *L. plantarum*, *L. helveticus*, and *S. thermophilus*

T3: Chickpea + Aquafaba inoculated with *L. plantarum* only

Each batch consisted of 1 kg of substrate mixture.

2.6 Inoculation and fermentation conditions

Each fermented batch was inoculated with 3% (v/w) probiotic culture under aseptic conditions. Fermentation was carried out in a controlled incubator at 37°C for 48 hours with relative humidity maintained between 70–80%. Anaerobic conditions were achieved using an anaerobic chamber with oxygen-scavenging sachets. pH was monitored every 12 hours using a calibrated digital pH meter. A non-fermented control sample consisting of mushroom and chickpea mixture without probiotic addition was prepared and stored under identical conditions.

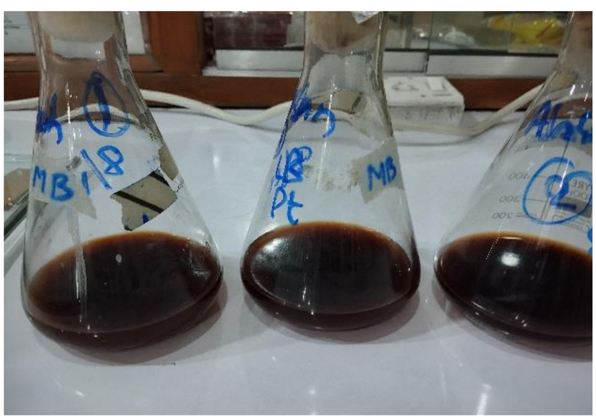

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Figure 2.1: Sample preparation for fermentation

2.7 Nugget formulation and processing

After fermentation, the substrates were blended to form a uniform nugget mixture according to the treatment formulations. Nuggets were shaped manually into nuggets (approximately 25 ±2 g each), coated with batter consisting of corn starch, salt, water followed by breadcrumbs, and deep-fried in refined sunflower oil at 180 ˚C for 4 mins until the nuggets turned golden brown and internal temperature of less than 75˚C obtained. After frying, the fried nuggets were cooled and packed in polythene bags and stored at −18°C for further analysis.

2.8 Proximate analysis

Proximate composition analysis was conducted using standard AOAC methods. Protein content was determined using the Kjeldahl method, fat content by Soxhlet extraction, crude fiber by gravimetric method, moisture content by oven-drying, and ash content using a muffle furnace by AOAC methods 925.10, 979.09, 920.39, 923.03 and 962.09 respectively. All analyses were performed in triplicate (AOAC International, 2023).

2.9 pH measurement

pH values of fermented and non-fermented samples were measured at 0, 24, and 48 hours. A 10% slurry was prepared by homogenizing 10 g of sample with 90 mL of distilled water, and pH was recorded using a calibrated digital pH meter.

2.10 Microbiological analysis

Microbiological safety was assessed using Total Plate Count (TPC) according to ISO 4833-1 (International Organization for Standardization [ISO], 2013). Ten grams of sample were homogenized with 90 mL sterile peptone water, serially diluted, plated on Plate Count Agar, and incubated at 30°C for 48 hours. Results were expressed as colony-forming units per gram (CFU/g).

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2.11 Sensory evaluation

Sensory evaluation was carried out using a panel of 15 semi-trained panelists. A 9-point hedonic scale was used to evaluate appearance, aroma, texture, taste, and overall acceptability of the nuggets. Samples were coded and presented randomly to panelists under controlled conditions.

2.12 Statistical analysis

All analyses were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was conducted to determine significant differences among treatments. Differences were considered statistically significant at $P < 0.05$.

Results

3.1 pH Analysis

The pH of a food product plays a vital role in determining its microbial stability, shelf life, enzymatic activity, and ultimately, consumer acceptability. In fermented food systems particularly those containing plant-based proteins like mushrooms and legumes pH reduction is both an indicator of successful lactic acid fermentation and a contributor to improved nutritional bioavailability and safety.

Table 3.1: pH analysis of different samples

Sample Type	Observed pH
Fermented Chickpeas	4.21 ± 0.03
Fermented Mushrooms	4.56 ± 0.02
Fermented Mixture (CMN 50:50)	4.66 ± 0.04

These findings are consistent with previously published studies on fermentation of legumes and mushrooms (Moorthi et al., 2022; Sharma et al., 2020).

3.2 Proximate Composition Analysis

Proximate analysis serves as a fundamental method in food science to evaluate the macronutrient composition of a product. It provides insights into the levels of moisture, protein, fat, fiber, ash (mineral content), and overall nutritional value critical for assessing dietary benefits, shelf-life potential, and consumer acceptance of any novel food product. In this study, fermented mushroom-chickpea protein nuggets (CMNs) were developed in three different ratios: 40:60, 50:50, and 60:40 (chickpea: mushroom), and each was evaluated for proximate composition. The data is summarized in the following table:

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Table 3.2: Proximate analysis of three different finished products

Proximate Parameter	CMN 40:60	CMN 50:50	CMN 60:40
Moisture	53.0	55.4	56.1
Fat	9.7	9.3	9.7
Fiber	7.01	7.03	6.06
Ash	5.2	5.5	5.92
Protein	23.0	24.1	26.8

Fiber is an essential macronutrient that supports gut health and reduces risks of chronic diseases. Among the three formulations, CMN 50:50 showed the highest fiber content (7.03%), slightly higher than CMN 40:60 (7.01%), with CMN 60:40 trailing at 6.06%.

Ash content represents the mineral residue left after combustion and reflects the presence of essential micronutrients like calcium, magnesium, iron, and zinc. CMN 60:40 showed the highest ash content at 5.92%, followed by CMN 50:50 (5.5%) and CMN 40:60 (5.2%).

Protein is the central focus of this study. The CMN 40:60 formulation (with higher mushroom content) achieved the highest protein content at 26.8%, followed by CMN 50:50 (24.1%) and CMN 60:40 (23%). However, fermented mushrooms especially *Pleurotus ostreatus* and *Agaricus bisporus* are dense in protein post dehydration, and fermentation further improves digestibility by denaturing antinutritional factors (Sharma et al., 2020; Wang & Zhao, 2023).

3.3 Microbiological Evaluation: Total Plate Count (TPC)

Microbial evaluation of food products is a cornerstone of food safety assurance, especially when fermentation is involved. In this study, Total Plate Count (TPC) was used to assess the microbial load of fermented mushroom-chickpea nuggets (CMNs) to determine product safety, shelf stability, and fermentation success. Since live bacterial cultures were intentionally incorporated to initiate fermentation, controlled microbial growth was expected (Sharma et al., 2020). The TPC was performed using serial dilution and plating methods in accordance with ISO 4833-1:2013 (ISO, 2013), which is widely recognized for aerobic mesophilic microorganism determination in food samples. All plates were incubated at 37 ± 1°C for 48 hours, and colony forming units (CFU/g) were counted manually.

Table 3.3: TPC of different batches

Sample/Batches	TPC (CFU/g)
Batch 1 (Mixed probiotic blend)	10^8 CFU/g
Batch 2 (2-strain culture)	10^8 CFU/g
Batch 3 (Single strain: L. plantarum)	10^9 CFU/g

The microbial load observed in all three batches is characteristic of active and successful lactic acid fermentation. Lactic Acid Bacteria (LAB), such as *Lactobacillus plantarum*, *L. delbrueckii*, and *Streptococcus thermophilus*, are known to proliferate rapidly under anaerobic and moderately

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warm conditions. The high CFU values (10^8–10^9 CFU/g) indicate efficient fermentation, dominance of beneficial probiotic strains, absence of contamination from spoilage/pathogenic organisms (based on morphology, no unusual colonies were detected). According to food microbiology standards, foods with LAB counts ranging between 10^7–10^9 CFU/g are considered microbiologically safe and functionally probiotic-rich. In fact, many commercial probiotic foods (e.g., yogurt, kimchi) fall within this range. The slightly higher count in Batch 3 is attributed to the exclusive use of *Lactobacillus plantarum* 299v, a robust strain with high acid tolerance and strong substrate affinity. Literature supports this; *L. plantarum* has demonstrated superior survival in low pH and high-fiber media compared to mixed strains (Sharma et al., 2020).

The Total Plate Count analysis confirms the microbiological success of the fermentation process, indicating the presence of safe, stable, and functional bacterial colonies. CMN formulations with robust LAB activity provide a food safety advantage and improved flavor profile while aligning with the need for chemical-free preservation methods. The microbial profile supports the commercial potential of these plant-based nuggets as nutritionally enriched and microbiologically safe alternatives to traditional meat-based snacks in Pakistan.

3.4 Sensory Evaluation of Fermented Mushroom-Chickpea Nuggets

This section evaluates the sensory characteristics of the fermented mushroom-chickpea nuggets (CMNs), focusing on six core attributes: appearance, aroma, texture, flavor, mouthfeel, and overall acceptability. A structured 9-point hedonic scale was used to assess consumer perception, ranging from 1 ("dislike extremely") to 9 ("like extremely"). Three formulations were tested i.e., CMN 40:60 (Chickpea: Mushroom), CMN 50:50, and CMN 60:40. All samples were thawed and shallow-fried using sunflower oil until golden brown. Once cooled to ~40°C, samples were served to panelists in coded trays under uniform lighting. Drinking water was provided between tastings to avoid carry-over effects.

Flavor is the most integrative sensory characteristic, combining aroma, taste, and aftertaste. CMN 50:50 offered a balanced umami and nutty flavor with mild acidity, attributed to lactic acid and amino acid derivatives from fermentation. CMN 40:60 had intense mushroom notes which were appreciated by some but rejected by others due to earthy bitterness. CMN 60:40 had a bland taste, indicating possible under-fermentation or masking of flavor by chickpea starches. Fermentation improved flavor depth in CMN 50:50 due to biochemical breakdown of complex compounds into flavorful peptides and acids. The cumulative evaluation across all parameters showed CMN 50:50 was most favored by panelists for its balanced appearance, flavor, and texture. CMN 40:60 was appreciated by mushroom lovers but less preferred by general panelists. CMN 60:40 scored lowest due to dryness and lack of distinct flavor.

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Table 3.4: Standard Deviation of three sample taken from total 15 evaluators

	CMN 40:60	CMN 50:50	CMN 60:40
Appearance	8.2	7.6	7.3
Aroma	8.2	8.3	6.6
Texture	8	7.8	6.3
Taste	8.4	7.4	6.3
Flavour Intensity	8.2	7.2	5.6
Overall Acceptability	8.3	7.7	5.6

The sensory evaluation demonstrates the clear advantage of fermentation in enhancing consumer acceptability of plant-based protein foods. The CMN 50:50 formulation showed the most balanced profile in terms of visual appeal, taste, texture, and aroma/1suggesting strong market potential. Future development could incorporate broader consumer testing, regional flavor adaptation, and packaging innovations for commercialization in the Pakistani market.

Discussion

4.1 Effect of Fermentation on pH and Product Stability

A consistent reduction in pH was observed in fermented formulations, reaching values in the approximate range of 4.21–4.66, which indicates effective lactic fermentation and acidification. This pH drop is technologically important for three reasons:

Acidification inhibits many spoilage and pathogenic bacteria and supports product safety and stability. This is consistent with the role of LAB fermentation described in the thesis background, where fermentation lowers pH and creates antimicrobial effects through organic acids and other inhibitory metabolites. Lower pH can shift protein solubility and protein–polysaccharide interactions, improving cohesiveness in a structured product like nuggets. This is particularly relevant because chickpea proteins and mushroom fibers create a matrix that responds to fermentation-driven biochemical changes (proteolysis and partial structural breakdown) (Grasso et al., 2022; Sharma et al., 2020; Webb et al., 2023).

LAB fermentation generates organic acids and volatile compounds that typically improve savory/sour balance and reduce raw “beany” or “grassy” notes from legumes. In practical terms, achieving a pH around ~4.2–4.7 supports the concept that fermentation can act as a “clean-label” stability tool alongside freezing and cold storage (Muniz & Du, 2021; Sharma et al., 2020).

4.2 Effect of Fermentation on Proximate Composition and Nutritional Quality

The fermented nuggets demonstrated a high protein level, with reported protein around 27.23% in fermented formulations. While fermentation does not create protein “from nothing,” it can increase

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measured or usable protein value through Proteolysis (protein breakdown to peptides and amino acids) which Improves digestibility and functional performance in the matrix. LAB-associated phytase activity reduces phytates, improving mineral bioavailability and potentially improving overall nutritional effectiveness of plant proteins. In addition, combining mushrooms and chickpeas is nutritionally strategic because the research rationale emphasizes complementary protein value and sustainability relevance for Pakistan (Boukid, 2021; Grasso et al., 2022; Sharma et al., 2020).

Moderate fat (reported ~8–9%) and fiber (up to ~7%) were retained in fermented formulations. This is important for two reasons which includes fat supports sensory quality (mouthfeel, flavor retention), which is critical in meat-analogue style products. Fiber supports health positioning and can improve texture by water binding, especially when combined with mushroom structural components (Mazumder et al., 2023; Nolden & Forde, 2023).

4.3 Microbiological Dynamics and Safety Interpretation

The fermentation process supported the proliferation of LAB while remaining within safe limits as described in the abstract summary. From a food safety perspective, this is an expected pattern: LAB dominate by rapidly lowering pH and competing with undesirable organisms. The thesis background also explains that fermentation can extend shelf life by producing bacteriocins, organic acids, and hydrogen peroxide, creating a hostile environment for spoilage/pathogens (Sharma et al., 2020).

A key discussion point is that microbial "increase" is not automatically "risk"—in fermented foods, the goal is controlled dominance of desirable microbes. Therefore, interpretation should focus on whether safety indicators (TPC limits, absence of pathogens if tested, and acceptable pH) support product stability.

4.4 Role of Upcycled Binder (Aquafaba) in Structure and Sustainability

Aquafaba was used as an upcycled binder to support sustainability and functionality. Its performance is mechanistically credible because aquafaba contains soluble proteins and polysaccharides that contribute to Emulsification and foaming**,** Gel-like binding**,** Moisture retention and cooking yield**,** which collectively support nugget integrity during shaping and cooking. From a sustainability standpoint, the binder choice directly supports the project's positioning around circular economy and reduction of food waste, aligning with the study framing that upcycled binders can replace costlier commercial binders and reduce losses across the food chain (Ali et al., 2025; Augustin et al., 2024).

4.5 Sensory Acceptability and Formulation Preference

Sensory evaluation indicated that the 50:50 mushroom to chickpea ratio was preferred in terms of flavor, texture, and overall acceptance (Mazumder et al., 2023; Moorthi et al., 2022; Yusof et al., 2021). This preference is explainable in product-technology terms which includes Higher mushroom fraction contributes umami flavor and a fibrous "meat-like" bite, improving palatability

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and reducing legume heaviness (Du et al., 2021; Muniz & Du, 2021; Muniz et al., 2022). Chickpea fraction supports body and binding but can contribute beany notes and density if too high; fermentation can reduce off-flavors, but the base ratio still strongly influences final sensory outcomes. Fermentation likely improved sensory acceptance through organic acid formation and flavor compound development, consistent with the thesis discussion of fermentation-driven taste improvement and off-flavor reduction (Can et al., 2024; Lang, 2020; Laureati et al., 2024; Michel et al., 2021; Siddiqui et al., 2022).

4.6 Relevance to Pakistan: Nutrition, Affordability, and Market Fit

The study's approach is strongly aligned with Pakistan's need for affordable, locally sourced protein options. The thesis establishes that protein malnutrition and food insecurity remain major challenges, and that innovation around local crops are needed to increase acceptability, especially among younger consumers (Gillani et al., 2022; Shabnam et al., 2023).

By using mushrooms and chickpeas ingredients highlighted as nutritionally valuable and locally relevant and combining them with fermentation and upcycled binders, the product concept fits three national priorities discussed in the research framing includes improved nutritional access (high protein, fiber, functional profile). Sustainable production (lower resource burden than animal proteins; reduced waste through upcycling). Consumer acceptability (optimized ratio and fermentation-driven sensory improvement) (Ali et al., 2025; Boukid, 2021; Rajpurohit & Li, 2023; Siddiqui et al., 2022).

4.8 Limitations and Practical Implications

Even when outcomes are positive, discussion should acknowledge realistic constraints that affect interpretation and scalability which includes Starter culture control, Fermentation outcomes can vary with inoculum, temperature, time, and raw material variability; strict process controls are needed for repeatability. While low pH supports stability, real-time shelf-life under frozen and chilled conditions should be supported with time-course microbiology, sensory, and texture measurements. Mixing, forming, and freezing behavior can change at industrial scale; binder performance must be verified in pilot-scale production. The results collectively support the central hypothesis that fermentation and upcycled binders can produce a nutritionally enhanced, acceptable, and potentially scalable plant-based nugget product. Fermentation contributed to acidification (pH ~4.21–4.66), improved functional/nutritional profile (including high protein), and supported sensory acceptance, while aquafaba served as a functional binder consistent with sustainability goals. Preference for the 50:50 mushroom: chickpea formulation indicates that optimizing ingredient balance is essential for consumer acceptance and product texture.

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