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Debittering and Masking Soy Peptides for Oral Consumption and Immune-Boosting Function

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ABSTRACT

Soy peptides (SPs) have been extensively studied with an emphasis on their immune-boosting effects and production-associated bitterness. This associated bitterness affects SPs' oral consumption despite their bioactivities. In this review, the immune-boosting functions, factors affecting bitterness, and consumers' perceptions of SPs are explored. Debittering/masking strategies to improve SP acceptability for oral consumption and formulation of SP-based food products are ultimately provided. It is established that SPs can contribute to immune functions despite different factors affecting bitterness perception by consumers, ranging from dietary and culinary to genetic and other components of the food matrix. Furthermore, SP bitterness levels can be reduced using a wide range of biophysicochemical debittering and masking methods. This review provides a unique and integrative perspective by linking the immune-boosting functions of SPs directly to the critical challenge of bitterness and its mitigation. Future studies focusing on highly efficient debittering techniques that support the immune-boosting effect, quality, and mouthfeel of SPs and SP-incorporated products are warranted.

1 | Introduction to Soy Peptides

In recent years, food scientists have increasingly focused on food-derived bioactive peptides due to their diverse health-promoting properties. Among these, soy peptides (SPs) have consistently garnered attention across in vitro, in vivo, and human studies [1–6]. The growing interest in SPs is largely attributed to their origin from plant-based legume proteins, offering a sustainable and vegetarian-friendly alternative to animal-derived peptides [7–10]. SPs are rich in free and essential amino acids (AAs) and exhibit a broad spectrum of biological activities and technofunctional properties [11–17]. Their demonstrated bioactivities, including anticancer, anti-inflammatory, and antimicrobial effects, have sparked a growing interest in their potential roles in chronic disease prevention, modulation of gut microbiota, and immune-boosting function [7, 16, 18–22].

The growing prominence of SPs has driven the development and application of advanced techniques aimed at enhancing their production and functional characterization. A wide array of innovative technologies is being employed, including pulsed electric field processing, enzymatic and chemical hydrolysis, and lactic acid, microbial, and ultrasound-assisted liquid-state fermentation [23–28]. These approaches are critical for optimizing yield, improving peptide bioactivity, and tailoring functional properties for specific health and industrial applications. SPs are

small protein fragments that generally undergo food processing/modification involving pH, heat, ultra-high-pressure, and gastrointestinal (GI) digestion by both specific and nonspecific proteases of the stomach, pancreas, and small intestine [22, 29]. SP production process affects their peptide composition and diverse functions, including the end product's texture, yield, and quality [30–32]. During both in vitro and GI digestion, soy protein is enzymatically hydrolyzed into a mixture of peptides of varying lengths and free AAs. To isolate and identify specific bioactive SPs from this complex mixture, further separation and purification steps are required. These processes typically involve techniques such as selective precipitation, membrane filtration, and various forms of liquid chromatography, which enable the enrichment and characterization of peptides with targeted biological activities [13].

SPs can survive gastric proteolysis and reach the small intestine, where their biological effects depend on molecular size, AA sequence, spatial arrangement, and net charge. Peptides enriched in cationic residues (Arg, Lys, and His), aromatic side chains, and branched-chain hydrophobic residues (Leu, Ile, Met, Val, and Ala) readily adopt amphipathic conformations, often stabilized by disulfide bonds. This amphipathicity enables strong electrostatic interactions with negatively charged bacterial membranes, culminating in membrane disruption and broad-spectrum antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Streptococcus mutans*, and *Propionibacterium acnes* [12, 13]. By limiting pathogen colonization, SPs indirectly reinforce mucosal immunity.

Paradoxically, the same hydrophobic and basic residues that confer bioactivity also stimulate human bitter taste receptors (TAS2Rs), imparting pronounced bitterness that can hamper consumer acceptance. Targeted debittering or masking strategies are therefore essential for the successful incorporation of SPs into functional foods, suitable for oral consumption. This review critically examines the immune-boosting functions of SPs, the process-induced bitterness of SPs, and strategies for debittering and masking bitter taste associated with SPs. This review provides a unique and integrative perspective by linking the immune-boosting functions of SPs directly to the critical challenge of bitterness and its mitigation. It critically examines the sensory-bioactivity paradox, uniquely integrates the current understanding of SP immunomodulation with a detailed analysis of the molecular basis of their bitterness, and subsequently provides a comprehensive evaluation of advanced debittering and masking strategies. By bridging these often-disparate fields, this work aims to present a cohesive framework for the development of sensorially acceptable, immune-boosting SP-based products.

2 | Immune-Boosting Function of SPs

A growing body of in vitro, animal, and human research indicates that SPs possess significant immune-boosting/immunomodulatory functions, mediated through direct interactions with key immune cells [33–36]. Upon absorption in the intestines, SPs can engage with innate immune sentinels such as macrophages and dendritic cells (DCs), as well as adaptive immune players such as T and B lymphocytes [37]. A critical mechanism involves the modulation of the nuclear factor-kappa B (NF-κB) signaling pathway, a master regulator of inflammation. Specific SPs have been demonstrated to inhibit the phosphorylation and degradation of IκBα, thereby preventing the nuclear translocation of the NF-κB subunit p65 [34]. This suppression leads to the downregulation of proinflammatory gene expression, resulting in decreased production of cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β). Concurrently, SPs can enhance the phagocytic capacity of macrophages, bolstering the innate clearance of pathogens [38].

The immunomodulatory influence of SPs extends to shaping adaptive immune responses through the regulation of T-cell differentiation and function. This is often mediated via the Janus kinase–signal transducer and activator of transcription (JAK–STAT) signaling pathway. For instance, SPs that promote a shift toward anti-inflammatory responses can upregulate the secretion of IL-10, which signals through the JAK1–STAT3 axis to suppress proinflammatory T-helper 1 (Th1) and Th17 responses [37]. Conversely, immunostimulatory peptides, such as the glutamine-rich analogs derived from glycinin, have been shown to increase the population of IFN-γ-secreting CD4+ T cells and CD49b+ natural killer (NK) cells [39]. This Th1-polarizing effect is indicative of STAT1 and STAT4 activation downstream of cytokines such as IL-12, highlighting the ability of different SPs to selectively engage distinct JAK-STAT pathways to fine-tune the immune response [35].

DCs, crucial antigen-presenting cells that bridge innate and adaptive immunity, are also key cellular targets for SPs. Bioactive peptides can influence DC maturation and cytokine secretion profiles, thereby directing subsequent T-cell priming. The mitogen-activated protein kinase (MAPK) pathways, including ERK, p38, and JNK, are often involved in this process [4]. SPs can modulate these kinases to alter the expression of surface costimulatory molecules (e.g., CD80 and CD86) and the production of polarizing cytokines. Furthermore, SP-induced changes in intracellular redox balance can indirectly influence these signaling cascades. By reducing the generation of reactive oxygen species (ROS), which are known activators of the NF-κB and MAPK pathways, SPs can exert an indirect anti-inflammatory effect, contributing to a more regulated immune environment [22].

Beyond direct intracellular signaling, SPs also mediate their effects by engaging specific cell surface receptors on immune cells. Although interactions with opioid receptors have been documented, leading to immunomodulatory outcomes, SPs can also act as ligands or modulators for other receptor families, such as Toll-like receptors (TLRs), which are pivotal in initiating innate immune responses [36]. Certain SPs may antagonize TLR4 signaling, thereby blocking the downstream activation of both NF-κB and MAPK pathways and preventing excessive inflammation driven by microbial components [10]. Therefore, modulation of TLR signaling (e.g., TLR4/NF-κB) by dietary peptides represents a significant mechanism for their anti-inflammatory effects. This multireceptor engagement, combined with the direct modulation of intracellular cascades, underscores the pleiotropic nature of SPs. The collective outcome, ranging from anti-inflammatory suppression to immunostimulatory enhancement, is dependent on the specific peptide sequence, its concentration, and the cellular context, ultimately

contributing to the overall immune-boosting phenotype observed in vivo [40]. Therefore, an ideal debittering strategy should not only block TAS2R binding in the mouth but also preserve the peptide's structural integrity for engaging with beneficial targets such as TLRs in the gut.

Secretory immunoglobulin A (sIgA) dominates mucosal surfaces, immunoglobulin M (IgM) provides the first systemic antibody response, and immunoglobulin G (IgG) is the principal serum antibody responsible for long-term humoral defense. Dietary interventions that elevate any of these classes are therefore regarded as supportive of host immunity. In aged rats, supplementation with SP-enriched nutrients markedly increased serum IgA, IgM, and IgG levels, reduced proinflammatory cytokines IL-1β and TNF-α, and normalized markers of T-cell activation [34]. The same study showed that SPs mitigated burn-induced hyperinflammation by modulating leukocyte counts and preventing the negative nitrogen balance typical of catabolic stress. Collectively, these findings suggest that SPs can both strengthen adaptive immune responses and temper excessive inflammation, supporting their potential use as adjuvant nutritional therapies during immune compromise or traumatic injury.

Bioactive peptides, including those derived from soy, can reinforce intestinal barrier integrity and influence mucosal and systemic immunity by interacting with epithelial, immune, and microbial cells (Figure 1). SPs may be absorbed in the intestines via several mechanisms, including carrier-mediated transport, transcytosis across enterocytes, and paracellular diffusion through tight junctions. Once absorbed, SPs can interact with immune cells such as T lymphocytes, B lymphocytes, DCs, and macrophages, particularly those expressing opioid or peptide-sensitive receptors, thereby stimulating the production of cytokines, immunoglobulins, and mucins [37]. SPs can also reduce ROS production, TNF-α, and NF-κB levels and downregulate NO/PGE_2 and iNOS/COX-2 in macrophages, while enhancing the phagocytic effect of peritoneal macrophages. These can contribute to intestinal barrier and anti-inflammatory effects, resulting in immune-boosting effects.

Leveraging this immune-modulatory premise, Maeda et al. [41] isolated an "immunoglobulin-production-stimulating factor" (IPSF) from soybeans that increased IL-6 and IL-10 mRNA in murine spleen cells, implying a peptide-based immunopotentiator. Yet the IPSF was obtained in low yield and modest purity, and its activity was demonstrated only in vitro; its precise sequence, stability during GI digestion, and in vivo efficacy remain unresolved. More compelling evidence was provided by Egusa and Otani [39], who hydrolyzed soy glycinin with *Rhizopus oryzae* peptidase R and purified a glutamine-rich peptide fraction that boosted cellular immune markers (CD8, CD11b, and CD49b) in C3H/HeN mouse splenocytes. Two synthetic glutamine-rich SP analogs, QQQQQQKSHGGR and KQGQHQQEEEEE, elevated IL-12-producing $CD11b^+$ cells; QQQQQQKSHGGR further increased $CD49b^+$ NK cells, IL-2- and IFN-γ-secreting $CD4^+$ T cells, and splenocyte cytotoxicity toward the K562 erythroleukemia line. These data underscore the immunostimulatory role of the glutamine-rich domain of the soy glycinin G4 subunit. Nonetheless, the absence of dose–response information and in vivo validation highlights the need for additional studies before these peptides can be firmly positioned as functional immunonutrients.

Early work by Tsuruki et al. [42] led to the isolation of a bioactive tridecapeptide, MITLAIPVNKPGR, designated "soymetide-13" from the α′ subunit of soybean β-conglycinin via trypsin digestion. This peptide was shown to stimulate phagocytosis in human neutrophils. A shorter derivative, "soymetide-4" (MITL),

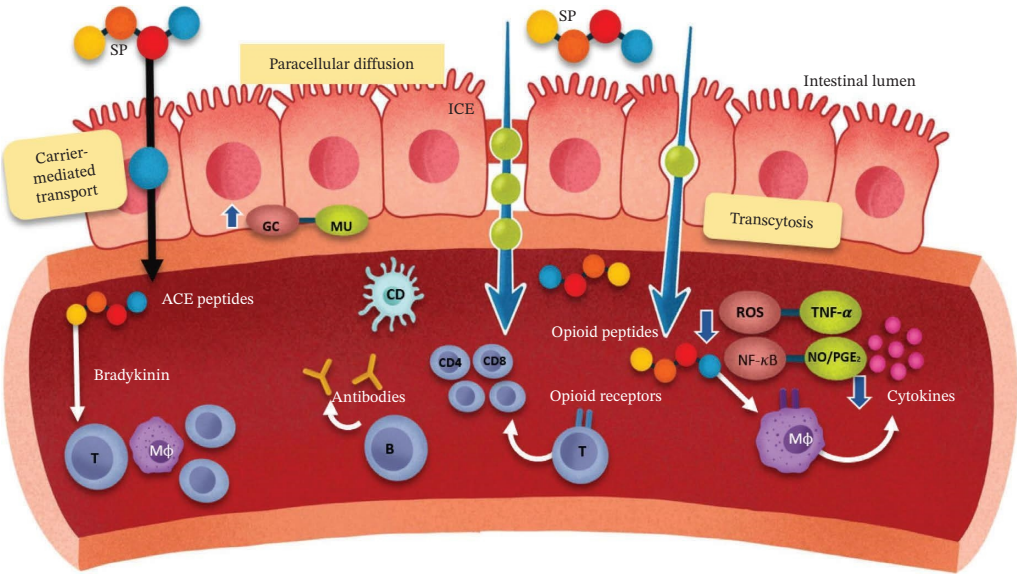

FIGURE 1 | Soy peptides and their potential mechanisms and interactions with various cells to enhance immune functions. Transportation through carriers, transcytosis, and paracellular diffusion are possible ways to absorb SPs in the intestines. SPs then interact with opioid receptors located on T lymphocytes or dendritic cells, B lymphocytes, and macrophages, thereby stimulating the production of cytokines, antibodies, and mucus. SPs can also reduce ROS production, TNF-α, and NF-κB levels and downregulate NO/PGE_2 and iNOS/COX2 in macrophages, while enhancing the phagocytic effect of peritoneal macrophages. These can contribute to intestinal barrier and anti-inflammatory effects, resulting in immune-boosting effects. SPs: soy peptides; ICEs: intestinal epithelial cells; MΦ macrophages; ROS: reactive oxygen species; NO: nitric oxide; iNOS: inducible nitric oxide synthase; PGE_2: prostaglandin-2; COX: cyclooxygenase; TNF-α: tumor necrosis factor-α; NF-κB: nuclear factor kappa-B; GCs: goblet cells; MU: mucus.

formed by C-terminal truncation, demonstrated even greater immunostimulatory activity, significantly enhancing TNF-α production in murine models. Subsequent work by Yimit et al. [33] expanded the understanding of SP immunomodulatory effects using near-infrared spectroscopy. SPs were enzymatically hydrolyzed from soy protein isolate (SPI) using microbial proteases (theroase, bioprase, and sumizyme FP) derived from *Bacillus* and *Aspergillus* species. In a clinical setting, healthy volunteers received 8 g of SPs dissolved in 200 mL of water. Results demonstrated modulation of cellular immune parameters, including changes in leukocyte, granulocyte, and lymphocyte subsets. Additionally, SP intake enhanced neurotransmitter activity and emotional hormone levels, suggesting a potential immunoneuroendocrine link. This study was particularly significant in demonstrating the immunostimulatory and neuromodulatory properties of SPs in humans, thereby strengthening their potential role in functional nutrition.

In a recent study on roosters, Wei et al. [43] demonstrated that dietary supplementation with SPs enhanced both immune and antioxidant capacities while promoting gut health. These benefits were attributed to improved intestinal structural integrity, reduced epithelial apoptosis, and increased microbial diversity, particularly the proliferation of beneficial gut microbiota. Notably, a dietary inclusion level of 0.45% SPs was identified as optimal for improving immune function, overall physiological health, and reproductive performance. These findings further substantiate the immunomodulatory potential of SPs and support their use as functional feed additives. However, one persistent challenge across both immune-related and nonimmune applications of SPs is their inherent bitterness, which can compromise palatability and consumer acceptance. Addressing this sensory limitation remains critical for the broader application of SPs in functional foods and animal nutrition.

3 | Bitterness in SP Production

Bitterness generated during the enzymatic or chemical hydrolysis of soy proteins remains a primary obstacle to the commercial use of SPs. Hydrolysis liberates hydrophobic AAs that concentrate within low-molecular-weight (MW) peptides, and these hydrophobic residues are largely responsible for the characteristic bitter taste [44, 45]. The resulting peptides often present dominant N-terminal α-amino groups, further accentuating surface hydrophobicity [46].

3.1 | Influence of Peptide Size and Sequence

Bitterness intensifies as peptide MW and chain length increase. In a model system, 4 kDa SPs were perceived as substantially more bitter than 1 kDa peptides [47]. The trend reflects a greater number of hydrophobic side chains able to interact with human TAS2Rs [48].

3.2 | The *Q*-Value Framework

The foundational explanation for these observations is Ney's *Q* hypothesis [49], which is a theoretical relationship framework between the bitter taste of peptides and hydrophobicity, quantifying average peptide hydrophobicity:

$$Q = \sum \frac{\Delta g}{n}, \quad (1)$$

where Δg is the transfer free energy and n is the number of AA residues.

The *Q* value is the peptide's average hydrophobicity and is defined as the ratio of the free energy changes in the transfer of AA side chains from ethanol to water to the number of AA residues in the peptide. If the *Q* value exceeds 1400 cal/mol, the peptide is almost certainly bitter, whereas peptides with *Q* values between 1300 and 1400 cal/mol have uncertain bitterness, and those lower than 1300 cal/mol have no bitter taste [50].

The *Q*-value concept underpins early predictive bioinformatic tools for peptide bitterness based on AA profile and chain length [51]. Its main limitation is applicability to peptides below ~6 kDa, beyond which conformational factors become dominant [49].

3.3 | Implications for SPs

By using the *Q* hypothesis, the bitterness of soy proteins/peptides could be predicted. Soybean glycinin and β-conglycinin possess intrinsic *Q* values of ~1540 cal·mol^{-1}, comparable to casein (1605 cal·mol^{-1}) and zein (1480 cal·mol^{-1}), indicating a high propensity to yield bitter peptides when the degree of hydrolysis (DH) is not carefully controlled [52]. Because the bitterness threshold decreases as the proportion of hydrophobic AAs rises, managing DH and sequence composition is critical for sensory quality [45].

3.4 | Consumer Relevance

Consumer acceptance of functional foods is notoriously low when pronounced bitterness is present. Consequently, understanding and predicting SP bitterness followed by targeted debittering strategies are essential steps in translating the immunomodulatory and other health benefits of SPs into palatable products. Seki et al. [53] hydrolyzed soy protein with *Bacillus licheniformis* alkaline proteases and reported intensely bitter peptides, scoring ≥ 5 plus (i.e., "very strong" bitterness) despite an average *Q* value of 1128 cal·mol^{-1}. The peptides responsible averaged 445.7 Da and 3.75 peptide length. Like other hydrolysates produced in the study (chain length 2.26–4.02 residues), they were enriched in hydrophobic side chains released into the aqueous phase during proteolysis. Such findings underline the limitations of *Q*-value predictions for very short peptides and have stimulated numerous debittering approaches, such as enzymatic solutions, fermentation of the end products, single or combined use of specific techniques (e.g., isoelectric precipitation, alcohol extraction, and gel separation/chromatography), and the use of transformative, modifying, or masking agents such as nucleotides, salts, and sugars [54, 55]. A detailed understanding of human taste perception is therefore essential for rationally selecting and optimizing these mitigation strategies in SP formulations.

4 | The Mechanism of Bitter Taste Perceptibility of Consumers

Humans experience bitterness through a well-defined sequence that integrates chemosensory recognition/substance identification, neurological signaling, and cognitive interpretation [56–59]. This perception begins when bitter compounds, including SPs, bind to the G protein–coupled bitter taste receptors, TAS2Rs, on

the tongue. This binding initiates a signaling cascade (detailed in Section 5) that culminates in the transmission of neural signals to the brain. Bitter peptides bind to members of the G protein–coupled receptor (GPCR) family, and at least 25 TAS2Rs are expressed on Type II taste bud cells of the tongue and palate [60]. Individual peptides can activate multiple TAS2Rs, and each TAS2R can respond to structurally diverse ligands, explaining the broad bitterness of hydrophobic SPs. Action potentials project to the nucleus tractus solitarius in the brainstem, relay through the thalamus, and terminate in the primary gustatory cortex (insula–frontal operculum complex). Higher-order integration with limbic circuits generates the conscious perception of bitterness and the affective aversion often associated with it [58, 61]. The perception of this bitter signal is not uniform across individuals but is influenced by a complex interplay of peptide properties, genetic makeup, physiological state, and environmental factors (Figure 2).

Numerous variables can influence the experience of bitterness, including the particular TAS2Rs activated, individual genetic variances in taste receptor genes, and the structural properties of the peptides, such as molecular size, hydrophilic property, and AA sequence [62]. Stronger bitter effects are often induced by smaller peptides, especially di- and tripeptides. The peptides with higher hydrophobicity tend to taste more bitter, and some specific AA sequences and compositions indicate that some AAs contribute more to bitterness than others [63]. For instance, di-, tri-, and tetraleucine showed bitterness intensities that were 8, 15, and 30 times stronger than monoleucine, respectively, with tyrosine and phenylalanine showing similar results in previous studies [64, 65]. The bitter taste of isoleucine is primarily mediated by TAS2R1 receptors [66]. In contrast, the bitterness of leucine peptides increases with chain length due to heightened hydrophobicity but is recognized by different TAS2Rs. For instance, Leu–Leu activates TAS2R1, whereas longer chains (tri- and tetraleucine) are potent agonists for TAS2R10 and TAS2R14, explaining their intensely bitter character [66–68]. The perception mechanism thus shifts from a single receptor for the AA to multiple receptors for its oligopeptides. The reason why specific individuals may be more sensitive to the bitterness of particular peptides than others can also be attributed to individual genetic variances in TAS2Rs, which can cause changes in how one perceives bitter flavors [69]. Furthermore, with extended exposure, the perception of bitterness might change over time and be influenced by other taste approaches. The study of this process is essential for formulating methods to alleviate peptide bitterness in food preparation and for creating more appetizing functional meals or nutraceuticals containing peptides [70, 71].

Important factors related to bitter taste perception are influenced by a complex interaction of environmental, physiological, and genetic variables that affect how people perceive and react to bitter peptides. Recognizing the differences in how various individuals and populations perceive bitterness is dependent on these attributes. Different people may feel different bitter tastes due to variances in receptor sensitivity and functioning caused by these genetic variants [72]. A critical factor is genetic variation in TAS2R genes. Polymorphisms can alter receptor sensitivity, expression, or downstream signaling efficiency. For example, polymorphisms in the TAS2R38 gene have been associated with the capacity to taste phenylthiocarbamide (PTC), 6-n-propylthiouracil (PROP), and similar chemicals, resulting in "taster" and "nontaster" phenotypes in the population. A subset of tasters perceives these compounds as intensely bitter, leading to their classification as "supertasters" [73, 74].

The perception of bitterness is additionally profoundly affected by physiological variables. Individual variations exist in the number and distribution of TAS2Rs and taste buds on the tongue and in other areas of the oral cavity, which might impact an individual's sensitivity to bitter peptides [75]. Moreover, salivary amount and flow rate might affect how bitter compounds and taste receptors interact, which can change how bitterness is perceived. Bitter taste perceptibility can also be impacted by age in taste perception, such as a gradual decrease in taste sensitivity with aging [76, 77].

Perceptibility of bitterness is also determined by both environmental and experiential conditions. Exposure to and tolerance

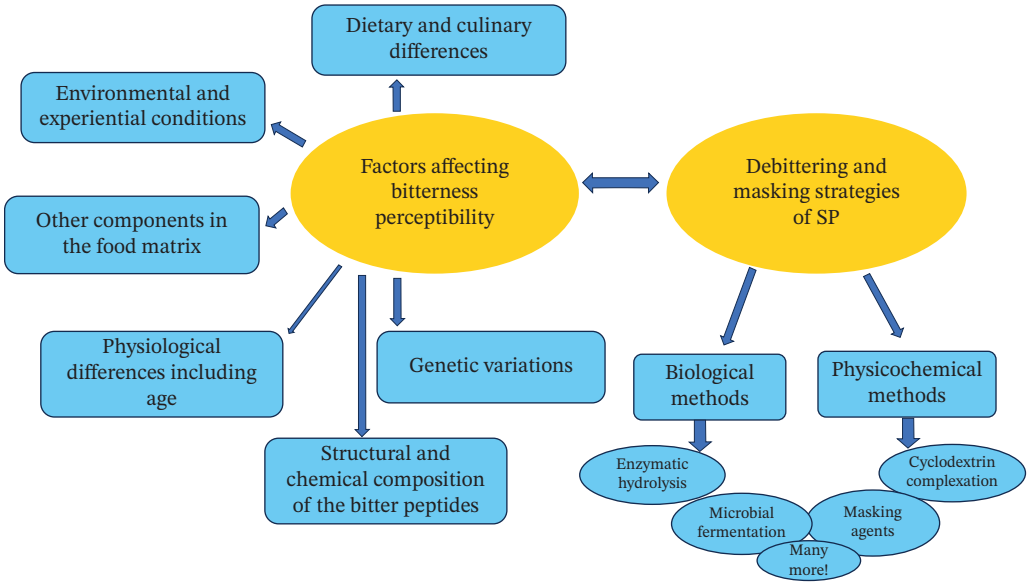

FIGURE 2 | Factors affecting bitterness perception and the debittering/masking strategies of soy peptides.

for bitterness can be attributed to dietary and regional culinary preferences. Consuming bitter foods and drinks regularly, including coffee or some vegetables, might eventually make bitterness easier to accept and even change how bitterness is perceived [78]. Furthermore, temperature, texture, and the presence of other flavoring substances such as sweeteners can all modify the bitter taste experience. As a result, the food matrix in which bitter ingredients are present can substantially impact how bitterness is perceived [79]. Bitter peptides' chemical composition and concentration significantly affect bitterness perception. Various bitter taste profiles and intensities can result from different bitter chemicals activating different combinations of TAS2Rs. Various chemicals and individuals have varying thresholds for sensing bitterness. Depending on the particular chemicals involved and the individual's unique perceptual traits, the temporal features of bitter taste perception, such as the onset, intensity, and duration of bitterness, can vary [80–82]. One or a combination of these factors applies to the bitter taste of SPs, which may be felt in functional products if not adequately masked or felt as a major peptide product if not debittered. Figure 2 relates the factors affecting bitterness perception to the debittering and masking strategies of SPs.

5 | Molecular Interactions With Taste Receptors

The molecular interactions between SPs and taste receptors reflect a complicated chemical cascade that informs the strength and quality of how bitterness is perceived. To analyze these pathways is important in the development of efficacious debittering practices. Bitter taste perception occurs via the TAS2R family, which is comprised of GPCRs embedded within taste bud cells. Although Section 4 covered the broader pathway of bitter perception, this section details the specific binding and activation mechanisms. Humans have 25 functional TAS2R subtypes, all with individual binding specificities and sensitivities. Although TAS2Rs are found primarily on Type II taste cells within taste buds, it has been shown recently that TAS2Rs are expressed in extraoral tissues such as GI tissues, respiratory epithelium, and immune cells, suggesting that TAS2Rs may serve functions in addition to and/or beyond just taste [83]. Beyond their role in oral bitterness perception, TAS2Rs are increasingly recognized for their extraoral functions, particularly within the GI tract. Activation of gut-expressed TAS2Rs by bitter compounds, including peptides, has been shown to stimulate the secretion of gut hormones such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), which influence satiety and metabolism [84]. Furthermore, TAS2Rs on immune and epithelial cells can modulate immune responses and contribute to maintaining gut barrier integrity. This dual role of TAS2Rs presents a fascinating paradigm: Although oral activation leads to sensory rejection, GI activation may contribute to the very health benefits, including gut-mediated immunomodulation, that functional peptides are designed to provide. This underscores the importance of debittering strategies that mitigate oral bitterness without compromising the peptide's ability to interact with extraoral TAS2Rs or other targets in the gut.

Structurally, the TAS2Rs, like other members of the GPCR superfamily of receptors, have the common scaffolding of 7-transmembrane domains with extracellular loops and transmembrane portions of the receptors forming the binding pocket for ligand recognition. Two important features of all GPCRs that are vital concerning how TAS2Rs recognize and perceive bitter taste are promiscuous binding sites (e.g., receptor can recognize many structurally different bitter compounds) and the diversity of individual receptor selectivity patterns that shape the overall bitter taste [85]. SPs utilize multiple molecular recognition mechanisms to bind with these bitter taste receptors. The hydrophobic peptides that have high amounts of aromatic AAs, such as tryptophan, phenylalanine, and tyrosine, and branched-chain AAs, such as leucine, isoleucine, and valine, have the greatest affinity for several TAS2Rs, especially TAS2R1, TAS2R14, and TAS2R39.

The binding can vary depending on the length, sequence, and 3D shape. Di- and tripeptides usually produce the most intense bitterness with the best luck of fitting the receptor pocket size. Although large peptides can show more or less bitterness depending on the structure to facilitate binding with the receptor, this is almost entirely sequence-dependent. Specific interactions may include hydrophobic interactions between hydrophobic patches of the receptor and aromatic peptide residues, hydrogen bonding between α-amide backbone atoms of the peptide and AA side chains of the receptor, and electrostatic interactions between charged AA residues. Besides, van der Waals forces contribute to the overall stability of binding [86, 87].

Upon binding of the peptide, the TAS2R receptors undergo conformational changes to activate coupled G proteins such as gustducin and other subtypes of G proteins. The activated G protein commences a downstream signaling cascade with the phospholipase C β2 (PLCβ2), which cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) to generate inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 then opens calcium channels, releasing calcium from the intracellular stores, which leads to the opening of calcium-sensitive monovalent cation channels, allowing depolarization of the cell, and subsequently depolarizing the Type I glial-like cells through gap junctions, which then releases ATP, stimulating the afferent nerve fibers that transmit the bitter signal to the brainstem [88, 89].

Peptide–receptor interactions exhibit classic pharmacological relationships between concentration and response, although important complicating factors exist. Many SPs have steep concentration–response curves, and making small increases in concentration could significantly increase bitterness perception. The threshold concentrations of peptides could vary widely from nanomolar to millimolar. Furthermore, peptide mixtures typical of soy hydrolysates may have additive, synergistic, or competitive effects. Some peptides may compete for receptor binding sites, which may reduce overall bitterness, whereas others may activate different receptor subtypes simultaneously to enhance the bitter response [90, 91]. Timing in the bitter peptide interaction with receptors is very important as the kinetics of the interaction will influence the temporal aspects of bitterness. Faster association rates are correlated with faster bitter onset, whereas slow dissociation rates will have longer-lasting bitterness. Some SPs even have particularly slow dissociation from certain receptors, allowing the bitterness to remain in the mouth long after swallowing. Another important factor related to timing and bitter taste is receptor desensitization. If someone is exposed to the bitter peptides for too long, it could result in desensitization due to receptor phosphorylation-mediated desensitization

mechanisms and could also explain adaptation-like phenomena that are noted during a prolonged period of consumption [92].

As introduced in Section 4, genetic polymorphism at the TAS2R genes creates individual differences in bitter peptide sensitivity. An individual single-nucleotide polymorphism could affect either affinity for the receptor, expression, or downstream signaler efficiency. An example of this would be TAS2R38; the individual variants could affect sensitivity to certain classes of peptides. Hence, contributing to the variability of findings would explain why some consumers would have more tolerance to bitter SP formulations. From a practical standpoint, the genetic variation of receptors suggests that packaging and product development strategies require a population-based approach to achieve debittering and consumer acceptance for populations that differ from a genetic standpoint [93–95].

6 | Debittering and Masking Strategies for SPs

Consumer acceptance of SP-enriched foods depends on suppressing their inherent bitterness while preserving nutritional and bioactive integrity. Current mitigation tactics broadly fall into biological and physicochemical methods. Sometimes, an integrative/multitechnical approach is warranted. In the biological methods, targeted enzymatic hydrolysis and controlled fermentations metabolize hydrophobic termini or generate flavor-modifying metabolites [96, 97]. In the physicochemical approaches, isoelectric precipitation, solvent (e.g., ethanol) extraction, membrane or gel chromatography, and micro- or nanoencapsulation selectively remove, adsorb, or entrap bitter peptides without damaging functional sequences. Integrative techniques involve sequential or combined treatments (e.g., enzymolysis followed by Maillard reaction and fermentation plus encapsulation) that frequently produce synergistic sensory improvements. Flavor modifiers such as nucleotides, mineral salts, polyphenols, and sweeteners can further mask any residual bitterness perceived via TAS2Rs [16, 45, 55, 98]. Each method has its strengths and limitations depending on the specific application, cost constraints, and desired outcome for improving palatability. Table 1 summarizes these strategies, highlighting their relative efficacy, cost considerations, and impact on peptide functionality.

6.1 | Biological Approach

Enzymatic hydrolysis is a principal approach that aims to degrade larger, bitter peptides into smaller, less bitter fragments. The hydrolysis conditions need to be optimized, and certain enzymes must be specifically selected for this approach. Enzymatic hydrolysis minimizes the taste of bitterness by targeting particular bitter AA sequences; nonetheless, excessive hydrolysis might result in the formation of unpleasant new flavors. Aminopeptidases can cleave off bitter AAs from the N-terminus of peptides to successfully reduce overall bitterness. In addition to improving the functionality and bioavailability of peptides, enzymatic hydrolysis may assist in solving taste-related challenges [99]. A few particularly bitter peptides, instead of numerous moderately bitter peptides, can cause bitterness. Although peptide linkages are formed to block the hydrophobic AAs at both ends, less bitterness is found in peptides containing hydrophilic AAs at the C- or N-termini as opposed to hydrophobic AAs [100]. Therefore, SP-based food products can be designed to incorporate hydrophilic AAs at the C- and N-termini of the target SPs.

In a recent study, soy protein hydrolysates (SPHs) were generated through two-step hydrolysis using Protex 6L and Protease A 2SD for 120 and 180 min, respectively [101]. The hydrophobic peptide fractions with hydrophobic AAs, including alanine, leucine, isoleucine, tryptophan, phenylalanine, and tyrosine, indicated a positive correlation with the bitterness of the SPH. To break down the bitter peptides generated, Protease A 2SD was used to cleave leucine and arginine residues from the N-terminus. Also, the $ABTS^{\cdot+}$ radical scavenging capacity (50.19%) and the ORAC (71.65 μmol/L TE/mg) values of the peptides revealed their antioxidant potential, which may contribute to immune functions. This study shows the possibility of using multienzyme hydrolysis as a viable method for producing SPH with minimal bitterness and possible antioxidant effects [101].

The study of Seo and colleagues [128] aimed to compare bitterness levels of SPs after hydrolysis of SPI by different types of commercial proteases (i.e., alcalase, neutrase, protamex, flavorsome, papain, and bromelain) with enzyme–substrate ratios of 1%, 2%, and 5% and varying DH at 50°C and pH 6.8 to 7.1. Their bitterness levels were compared using taste dilution analysis. The dilution factor, which is the taste difference between a diluted sample and two blanks, was applied. Results showed the bitterness level to be getting stronger as the DH increased for all proteases used. At all DH ranges, flavourzyme hydrolysates had the lowest taste dilution factor, whereas at the same DH, alcalase presented the highest taste dilution factor, followed by neutrase. The taste dilution factor of hydrolysates at 10% DH was 0 for flavourzyme, 4 for protamex, and 16 for alcalase [8, 128]. Although the study suggested an alternative evaluation of SP bitterness to the hedonic scale sensory evaluation, it also complements *Q*-value evaluation and shows that enzymatic hydrolysis is a useful method of reducing bitterness in SPH/SP.

Proteases derived from soybean seedlings were applied to soybean seeds, which were grown for 1–10 days, to investigate their debittering effect on the obtained SPH [46]. The maximum protease activity (130 U/g) was observed in the seedlings on the sixth day. The protease activity rose to 2675 U/g at pH 5.5°C and 50°C after purification. The SPH generated by alcalase 2.4 L had its bitterness score reduced from 3.45 to 0 in 3 h by the proteases as the DH of the SPH elevated from 11.87% to 15.61%. Also, the SPH demonstrated an antioxidant activity [46]. This study also supports the concept of bitterness removal of SPs while maintaining immune-boosting functions through enzymatic hydrolysis.

Other than enzymatic hydrolytic means, fermentation techniques have been applied to reduce bitterness levels in SPs. For example, the debittering influence of carboxypeptidase and proteases from *Bacillus subtilis* ACCC 01746 on soybean meal was investigated using solid-state fermentation [104]. The proteases predominantly hydrolyzed the soybean meal protein into long-chain peptides with a slight bitterness. An extended fermentation time of 8–16 h increased bitterness due to carboxypeptidase synthesis. After 16 h, more carboxypeptidase is produced by the strain, which significantly decreased bitterness from 5 to 0. Significant debittering is achieved when carboxypeptidase is present because it cleaves four AAs, that is, phenylalanine, alanine, tyrosine, and leucine, at the C-terminus of

Table 1 | Various strategies for the debittering of peptides to improve their palatability.

Strategy	Description	Strengths	Limitations	References
Enzymatic hydrolysis	Uses specific enzymes such as proteases, alcalase, and papain to break down bitter peptides into smaller, nonbitter components	• Reduces bitterness effectively • Enhances digestibility and bioavailability	• Overhydrolysis can negatively impact texture • Requires precise control and optimization	[99–101]
Addition of flavor maskers	Adds sweeteners and natural or artificial flavor enhancers to mask the bitter taste of soy peptides	• Simple and cost-effective solution • Immediate improvement in palatability	• Does not eliminate bitterness • Can add extra calories • May alter the natural flavor profile	[55, 102, 103]
Fermentation	Involves microbial fermentation such as *Lactobacillus* to modify and reduce bitter peptides, often improving flavor profile and enhancing probiotic benefits	• Reduces bitterness naturally • Can improve nutritional and functional properties such as probiotics	• Long fermentation time • Inconsistent results based on strain or conditions	[46, 104]
Bitter blockers	Uses compounds that block bitter taste receptors, making bitterness less perceptible to consumers	• Directly addresses the sensory perception of bitterness • Effective at low doses	• May not be effective for all types of bitterness • Adds synthetic ingredients to the product	[98, 105]
Encapsulation	Encapsulates bitter peptides in a coating, such as lipid-based systems, to prevent interaction with taste receptors	• Masks bitterness effectively • Can enhance delivery of bioactive compounds	• Expensive process • Can change the texture and mouthfeel of the product	[21, 106, 107]
Cyclodextrin complexation	Cyclodextrins form complexes with bitter compounds, trapping them and preventing interaction with taste receptors	• Selectively reduces bitterness without affecting other sensory properties • Minimal impact on nutritional value	• Cost of cyclodextrins • May require additional process optimization	[108–112]
Peptide filtration	Physically filters out bitter peptides, separating them from nonbitter peptides based on size or molecular weight under ultrafiltration or nanofiltration	• Effective at selectively removing bitterness • Maintains nutritional and functional properties	• High cost of filtration equipment • Time-consuming process	[113–117]
Lipid-based delivery systems	Incorporates bitter peptides into liposomes or emulsions to mask bitterness and improve palatability	• Improves mouthfeel and reduces bitterness • Can enhance bioavailability	• Adds fat content • More complex manufacturing process • Can affect overall texture	[118, 119]
Aroma enhancement	Intensifying desirable aromas, such as vanilla and chocolate, can mask the perception of bitterness	• Easy to implement • Can improve overall sensory appeal	• Does not eliminate bitterness, only distracts from it • May not work for all consumer preferences	[120–122]
Protein cross-linking	Cross-links bitter peptides with other proteins such as transglutaminase to alter their structure and reduce bitterness	• Maintains functional and nutritional properties • Reduces bitterness effectively	• Complex manufacturing process • Expensive enzyme treatment	[16, 123]

(Continues)

TABLE 1 | (Continued)

Strategy	Description	Strengths	Limitations	References
Bitter peptide removal via adsorption	Uses adsorption materials, such as activated carbon, to selectively bind and remove bitter peptides	• Efficient removal of bitterness • Does not alter other properties of the peptides	• Can be costly and require extensive filtration • Loss of some functional peptides	[45, 124]
Blending with other proteins	Mixes soy peptides with other proteins such as whey protein to dilute bitterness and improve taste	• Simple and cost-effective • Improves overall nutritional value	• May alter the protein content and functional properties • Does not fully eliminate bitterness	[98, 105, 120]
Thermal processing	Uses heat to reduce bitterness by altering the structure of bitter peptides	• Simple process • Can be combined with other methods	• High temperatures may degrade nutritional value • Risk of off-flavors developing	[46, 102, 125, 126]
Addition of umami or savory compounds	Incorporates umami-tasting compounds such as monosodium glutamate (MSG) or yeast extracts to balance and mask bitterness	• Effective at balancing flavors • Improves overall taste perception	• Use of MSG may be undesirable for some consumers • May not fully eliminate bitterness	[48, 76, 121, 127]

Abbreviation: TAS2Rs, bitter taste receptors.

the bitter SPs in SPH. Moreover, 62.81% of hydrophobic AAs were found in the bitter SPs, whereas 16.22% present in the fermented soybean meal hydrolysate pool was identified as free AAs [96, 104].

Enzymatic hydrolysis and fermentation methods may be individually used or combined with other methods to debitter SPs. One technique is transglutaminase (TGase) cross-linking. In a recent study, alcalase enzymatic hydrolysis was combined with TGase cross-linking to generate hypoallergenic and less bitter SPH [46]. Under optimal heterogeneous enzymatic procedures, SPHs with 2.5%–10.0% DH were produced, and TGase polymerized them to produce varying peptide compositions. Observations showed that the polypeptides in SPHs were reorganized by glutaminyl transformations due to posthydrolysis cross-linking. The bitterness of SPH was significantly decreased after TGase treatments due to the masking of bitter peptides and free AAs produced. By adding TGase, however, the residual allergenicity of SPH in the serum of soybean-allergic patients was not affected, and neoallergens were not generated [46]. Therefore, the combination of enzymatic hydrolysis (in this case, using alcalase) and TGase cross-linking can be an effective strategy to enhance bitterness masking in SPs.

The fragmentomic and combination techniques, including in vitro and silico trials, are also useful strategies. In a study, these methods were used to evaluate potential bitterness in SPs [105]. Five main peptide sequences originating from soybeans were hydrolyzed using Proteinase K, papain, ficin, and bromelain as part of the bioinformatic-assisted analysis. The BIOPEP-UWM database was used to examine bitterness. Reversed-phase high-performance liquid chromatography–mass spectrometry (RP-HPLC-MS/MS) for peptide characterization and soy protein concentrate hydrolysis and RP-HPLC for proteolytic analysis were used to verify the results under optimal conditions. The identification of SPH revealed differences between in vitro and in silico results. SPH had nine bitter (parent) peptides, namely LSVISPK, DVLVIPLG, LIVILNG, NPFLFG, ISSTIV, PQMIIV, PFPSIL, DDFFL, and FFEITPEK [105]. Oligopeptides were prepared from the soybeans using tripeptidase and alkaline protease, in which the amount of free AAs rose considerably from 22.7% to 47.4%, especially those associated with umami flavor and sweet taste. This led to an 8.7% reduction in bitterness level, indicating that the soy oligopeptides generated had less bitterness [105, 129]. The findings demonstrated a considerable improvement in the bitter taste and other functional characteristics of SPs using the aforementioned techniques. Apart from the biological and bioinformatic strategies described, other physicochemical debittering and masking methods may be employed.

6.2 | Physicochemical Approach

The apolar groups present in soy protein's tridimensional configuration are exposed during hydrolysis and can result in a bitter taste of the derived SPs, despite exerting nutritional and physiological effects. Cyclodextrin addition, microencapsulation, and adsorption by activated carbon have all been utilized to mitigate the SP bitterness in food products [130]. Cyclodextrins have several applications in foods, including decreasing the bitterness of tannins and protein hydrolysates. To alleviate undesirable tastes, by constructing chemical complexes with undesirable flavor molecules and by binding to the carrier proteins of taste receptors, cyclodextrins may eliminate bitterness [131]. Debittering of SPs and other short food peptides and AAs could be attained by α-cyclodextrins and β-cyclodextrins [108]. By using response surface methodology to debitter and optimize the reducing power of SPH and SPs, a group of researchers [109] showed that 2% β-cyclodextrin introduced to SPH at 38.50°C and incubated for 12 min could optimally achieve SPs with the lowest level of bitterness and highest reduction power. Under these conditions, the bitterness level was reduced to 0.290, whereas the reducing power was 0.453 at $OD_{700\,nm}$. However, both values were below the detection threshold of bitter taste.

Bitter AAs, also known as taste–bitter AAs, are a group of AAs that contribute to a bitter taste when they are present in food or beverages. Examples are phenylalanine, tryptophan, proline, and tyrosine. They can interact with the nonpolar positions of the α- and β-cyclodextrins. In sensory studies, flavor and taste differences between the solutions comprising AAs and α- or β-cyclodextrin complexes were observed [110, 111]. When α- and β-cyclodextrins were introduced to SPH, a decrease in bitterness was observed. The bitter flavor and taste of SPH and AA were eliminated by α-cyclodextrin, indicative of its debittering effect on SPs incorporated in low-pH beverages. The results also suggested that β-cyclodextrin is a potential bitterness-masking ingredient in novel functional food products because it could reduce the perception of bitterness of SPs by 90% when 5% β-cyclodextrin was applied [110, 111]. Another group of researchers employed α-, β-, and γ-cyclodextrins in the debittering effort by interacting them with SPs after alcalase hydrolysis of soybean meal at 1% protein/protein concentration [112]. An electronic tongue system analysis revealed that the addition of 1.5% and 2.0% (w/w) α- and β-cyclodextrins to the pool of hydrolysates caused a significant difference compared to the control hydrolysates. In a bitterness rating test, the treatment effectively lowered bitterness compared to the control samples. The difference could also be recognized by untrained tasters [112]. The results displayed that this is an effective strategy to disguise bitterness in SPs.

Sweet compounds offer another means of bitterness masking. For instance, sweet compounds including xylitol, sucrose, α-cyclodextrin, and maltodextrin and their combinations were applied to mask bitterness in an enzyme-treated SPI in water and bread models [102]. Xylitol, sucrose, and maltodextrin all significantly decreased bitterness in the aqueous model, but α-cyclodextrin did not. Sucrose and xylitol significantly lowered bitterness compared to maltodextrin. Bitterness reduction was not impacted by any interactions between the taste-masking agents. In contrast to the bread model, the aqueous model demonstrated a higher bitter-masking impact. In both models, α-cyclodextrin and maltodextrin had comparable bitter-masking results, but xylitol and sucrose had different effects on bitterness reduction [102]. The findings showcased these agents as bitterness maskers in SPs incorporated in bread.

The primary umami-active component of Korean soy sauce and its effect on individual perception and TAS2Rs were also reported by Kim et al. [127]. The flavor is associated with the free AA and glutamic acid-enriched oligopeptides in a < 500 Da MW fraction. In human TAS2Rs, that is, hTAS2R43 and hTAS2R46 cells, the fraction mitigated bitterness and significantly inhibited the

TABLE 2 | Biological and physicochemical debittering and masking of soy hydrolysates and peptides.

Enzymes and reagents	Procedures	Significant results	References
Biological approach			
Protex 6L and Protease A 2SD	Two-step hydrolysis: Protex 6L (2 h) and then Protease A 2SD (3 h). Protease A 2SD cleaved Leu and Arg at the N-terminus	Positive correlation between hydrophobic peptide fractions and bitterness. Effective bitterness reduction via multienzyme hydrolysis. Hydrolysates demonstrated antioxidant potential	Tong et al. [101]
Alcalase, neutrase, protamex, flavorsome, papain, and bromelain	SPI hydrolyzed by different proteases at varying DH levels, with E/S ratios of 1%, 2%, and 5%. Bitterness compared using taste dilution analysis	Bitterness intensity increased with DH. Flavourzyme hydrolysates had the lowest bitterness. Alcalase hydrolysates were the most bitter at the same DH	Seo et al. [128]
Proteases from the 6th-day soybean seedlings and alcalase 2.4 L	Proteases extracted from soybean seedlings. Used to debitter SPH produced by alcalase	Seedling proteases reduced the bitterness score from 3.45 to 0. Increased the DH from 11.87% to 15.61%. Antioxidant activity was maintained	Zhang et al. [46]
Carboxypeptidase and proteases from *Bacillus subtilis* ACCC 01746	Solid-state fermentation of soybean meal. Carboxypeptidase activity increased with fermentation time (up to 16 h)	Carboxypeptidase cleaved Phe, Ala, Tyr, and Leu from the C-terminus. Reduced bitterness from 5 to 0. Converted bitter peptides into free AAs	Yin et al. [104]
Transglutaminase and alcalase	Combined alcalase hydrolysis with TGase cross-linking. Applied to SPH with 2.5%–10.0% DH	TGase treatment significantly reduced bitterness. Masked bitter peptides and free AAs. Reduced allergenicity without creating neoallergens	Zhang et al. [46]
Proteinase K, papain, ficin, and bromelain	Five soy peptides hydrolyzed by four different enzymes. Bitterness predicted in silico (BIOPEP-UWM) and verified in vitro (RP-HPLC-MS/MS)	Identified nine bitter parent peptides in SPH (e.g., LSVISPK and DVLVIPLG). Discrepancies noted between in silico and in vitro results	Iwaniak et al. [105]
Tripeptidase and alkaline protease	Soy oligopeptides prepared using a combination of endo- and exopeptidases	Increased free umami and sweet AAs (22.7%–47.4%). Reduced bitter value by 8.7%	Wu [129]
Physicochemical approach			
α-Cyclodextrin	Response surface methodology used to optimize β-cyclodextrin addition to SPH	Optimal conditions: 2% β-cyclodextrin, 38.5°C, 12 min. Achieved the lowest bitterness (0.290) and the highest reducing power	Hou et al. [109]
α- and β-cyclodextrins	Cyclodextrins complexed with bitter amino acids and SPH. Sensory analysis of complexes vs. controls	α-Cyclodextrin eliminated bitter taste in low-pH beverages. 5% β-cyclodextrin reduced bitterness perception by 90%	Linde et al. [110, 111]
α-, β-, and γ-cyclodextrins, alcalase® 2.4 L	Cyclodextrins (1.5%–2.5% w/w) added to alcalase-hydrolyzed soybean meal. Bitterness assessed via electronic tongue and sensory panel	1.5% and 2.0% α- and β-cyclodextrins significantly reduced bitterness. Difference was detectable by untrained tasters	Monge Neto et al. [112]
Xylitol, sucrose, α-cyclodextrin, maltodextrin	Sweet compounds tested as masking agents in aqueous and bread models containing enzyme-treated SPI	Xylitol, sucrose, and maltodextrin reduced bitterness in the aqueous model. α-Cyclodextrin was ineffective. Masking effect was more pronounced in the aqueous model than in bread	Bertelsen et al. [102]
Umami-active components from Korean soy sauce	Fractionated Korean soy sauce to isolate umami-active components (< 500 Da). Tested on human TAS2R43 and TAS2R46 cells	Umami fraction mitigated bitterness and inhibited caffeine-induced calcium response in TAS2Rs. Flavor attributed to free AAs and glutamic acid-enriched oligopeptides	Kim et al. [127]
Sodium chloride	Sensory analysis of Korean fermented soybean paste with 8%–20% NaCl during early aging (0–2 months)	Aging enhanced mouthfeel, astringency, and bitterness. 8% NaCl samples were less acceptable to consumers due to a distinctive (likely more pronounced) flavor profile	Kim et al. [40]

Abbreviations: AAs, amino acids; DH, degree of hydrolysis; E/S, enzyme–substrate ratio; RP-HPLC-MS/MS, reversed-phase high-performance liquid chromatography–mass spectrometry; SPH: soy protein hydrolysate; SPI, soy protein isolate; TAS2Rs, bitter taste receptors; TGase, transglutaminase.

intracellular calcium ion response caused by caffeine [127, 132]. From this study, the low-MW SP fraction can regulate the function of human TAS2Rs to enhance food taste.

Bitterness can also be effectively reduced by adding sodium chloride (NaCl) to SPs. Kim et al. [40] investigated the influence of 8%, 12%, 16%, and 20% NaCl concentrations on Korean traditional fermented soybean paste production and the sensory characteristics and consumer acceptability from 0 to 2 months of the early aging stage. As the concentration of NaCl in the soy paste increased, the concentrations of sodium and magnesium also increased. Mouthfeel characteristics, astringency, and bitterness became substantially enhanced by the aging process. However, 8% of NaCl samples displayed a distinct pattern of modifications to early aging perception. In contrast to the samples containing 12% and 16% NaCl, consumers found the 8% NaCl samples less acceptable due to their distinctive flavor characteristics [40]. In Table 2, biological methods including enzymatic hydrolysis and microbial fermentation are shown as effective in masking bitter peptides to improve the overall taste and product value. Physicochemical methods including masking agents and cyclodextrin complexation either block or remove the bitter components. Each method has its advantages and drawbacks, and the choice of approach depends on the desired balance between efficiency and sensory quality in the final product.

7 | The Effect of Bitterness on Consumer Acceptance

Consumer acceptance is easily the largest hurdle in promoting SPs as a functional food inclusion, in large part due to the complex physiological, psychological, and market interaction issues related to bitterness that can affect the viability of products. The human perception of bitterness arose from a naturally evolved protective mechanism against toxic compounds, which renders bitterness instinctively unaccepted. The human tongue has about 25 bitter taste receptors, TAS2Rs, that can identify the AAs and peptide fragments often found in bioactive SPs. Bitter taste perception is triggered by hydrophobic AAs such as phenylalanine, leucine, and tyrosine, which are abundant in soy protein hydrolysates that induce a bitter taste, often at low concentrations. This physiological reaction creates an immediate negative first perception that supersedes any ability to cognitively value the health benefits [133]. Research has shown that the bitter taste is by far the major sensory characteristic for consumer rejection of protein hydrolysates. To put this in perspective, studies have shown the bitterness detection threshold for SPs is in the range of 0.1–0.5 mg/mL, which is well below the range of 5–20 mg/mL required for functionality as immune boosting. The level of consumer acceptance rapidly declines at certain levels of intensity (rate of bitterness), with rejection rates as high as 70%–90% of unmasked SP products [5]. To make matters worse, bitterness also has a duration element to consider—bitter peptides have been shown to exhibit a delayed onset profile but long-lasting duration—that is, they leave behind a lingering aftertaste that lasts longer than the consumption event. The nature of the lingering taste, not based on the actual time consumption event, contributes to long-term memory formation and results in a lower likelihood of a consumer wanting to consume something again, which is especially problematic for health and wellness products that are required to be consumed daily to realize health and wellness benefits [134].

Consumer resistance to bitterness directly leads to market failure of functional food products. Because increasing peptide concentrations may raise bitterness in tandem with bioactivity, it presents product developers with a small margin of commercial relevance. Research suggests taste is the most significant driver of purchase when it comes to functional foods, with taste consideration often superseding health benefits in the decision-making process of consumers. The problem is especially severe with the target consumer segments that stand to benefit most from immune-enhancing peptides, namely elderly consumers and health-forward adults. Adult consumers often demonstrate increased bitter sensitivity due to biological or medical reasons, which potentially amplifies their perception of bitter [135]. Bitterness not only triggers immediate sensory rejection, but it also creates negative psychological associations, which go beyond taste. In many cases, consumers associate bitter-tasting flavors with poor-quality products, artificial additives, or processing defects. This bias may weaken marketing claims relating to naturalness and health claims, ultimately creating cognitive dissonance that constrains purchase consumption choices and trust in the presentation of the brand. Similarly, hedonic effects of bitterness have a negative impact on consumption and compliance. Products marketed for daily consumption as supplements or functional foods may not only require tolerance but also often require a positive hedonic experience to create long-term adherence to the dosing regimen recommended [136]. The influence of bitterness on consumer acceptance underscores the strategic importance of both debittering technologies and masking approaches in SP product development. To successfully commercialize an SP, it will need to either limit bitterness compound formation during processing, remove bitterness postproduction, or mask or eliminate bitter sensations as part of the formulation strategy. The economic case is very clear. If these bitterness barriers can be successfully navigated, SPs have the potential to become functional food components that fit the regular consumer's food choices; their future public health impact will depend on the scale that would be attained [137].

8 | Conclusions

Process-induced bitterness remains the principal sensory barrier to the widespread incorporation of SPs in functional foods. A spectrum of biological interventions (e.g., targeted enzymatic hydrolysis and controlled fermentations) and physicochemical measures (e.g., TGase cross-linking, selective precipitation, encapsulation, and flavor modulators such as salts or sweeteners) can individually or in combination attenuate bitterness without compromising peptide bioactivity. Nevertheless, bitterness perception is not uniform: It is modulated by peptide sequence and conformation, coingredients in the food matrix, culinary practices, physiological state, and interindividual genetic variation in TAS2Rs. Future work should prioritize integrated debittering platforms that couple advanced biocatalysis, membrane fractionation, and encapsulation to achieve near-complete removal or masking of hydrophobic peptides at an industrial scale; structure–function mapping to identify minimal peptide motifs that retain immunomodulatory efficacy while remaining below bitterness thresholds; personalized sensory approaches that account for genetic polymorphisms in bitter–taste receptors, enabling product optimization for diverse consumer groups; and in vivo validation linking debittering strategies to maintained or

enhanced immune benefits, ensuring that technological modifications do not diminish the health value of SPs. By integrating sensory science with biochemical and process engineering advances, the next generation of SP-enriched foods can deliver both robust immunological benefits and broad consumer appeal.

Author Contributions

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