Food Science of Animal Products

Review Article

Research advances in kokumi peptides: a comprehensive review from flavor mechanisms to food industry applications

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Abstract: Kokumi taste, a sensory attribute that enhances flavor complexity, continuity, and mouthfulness without being a basic taste, has gained significant scientific attention. This review synthesizes advances in kokumi peptide research, focusing on their flavor-enhancing mechanisms, receptor interactions (notably calcium-sensing receptors), natural sources, and food applications. We critically evaluate global research progress and key debates, such as receptor synergy mechanisms and limitations of current flavoring mechanism. Future directions emphasize establishing a global kokumi peptide database, developing artificial intelligence-driven prediction platforms, improving *in vivo* validation, and optimizing industrial-scale production. This review aims to provide a theoretical foundation for food flavor science and to facilitate the integration of kokumi peptides into the development of functional foods and flavor enhancers.

Keywords: kokumi peptides; calcium-sensing receptors; flavoring mechanism; receptor recognition; flavor enhancement

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1 Introduction

Kokumi is a sensory concept distinct from the five basic tastes, defined not by a specific flavor quality of its own but by its capacity to modify and enhance the overall gustatory profile through characteristics commonly described as "mouthfulness", "continuity", and "thickness". Unlike sweet, sour, salty, bitter, and umami, which are perceived as independent taste modalities elicited by specific receptors, kokumi substances—often γ-glutamyl peptides—act primarily as taste enhancers that amplify the intensity and duration of these basic tastes without imparting a strong independent taste at typical culinary concentrations. This distinction categorizes kokumi as a modulatory phenomenon that increases the complexity and roundness of food flavor, rather than a standalone primary taste sensation[1].

Amid the ongoing rise in consumer demand for health-conscious dietary options, functional foods characterized by reduced salt, low fat, and high protein content have emerged as prominent areas of research. Nevertheless, these products frequently encounter challenges related to bland taste profiles and inadequate flavor complexity. Consequently, a significant challenge confronting the global food technology sector is how to preserve or augment the palatability of such foods without increasing sodium levels[2]. In this context, the sensory attribute known as "kokumi", which has the capacity to enhance overall flavor perception, has increasingly attracted scientific attention. Plant-based foods, including soy-derived products and pea protein beverages, commonly exhibit challenges related to undesirable off-flavors, such as beany and bitter notes, as well as weak flavor profiles[3–6]. Moreover, the research on the application technology of kokumi peptides from natural sources as food ingredients in compound seasonings helps enrich the flavor of compound seasoning products and also plays a role in cleaning labels, thus attracting widespread attention. Consequently, there is a pressing demand for effective strategies to modulate and improve their flavor characteristics. Kokumi peptide have garnered considerable interest due to its dual functionality: it not only amplify umami synergistic effects, but also effectively suppress unpleasant flavors.

Kokumi peptides act on non-traditional taste pathways, such as calcium-sensing receptors (CaSR) and G protein-coupled receptor class C group 6 member A (GPRC6A), broadening our understanding of "non-classical taste signals", which has further deepened insights into the mechanisms of taste perception. Yamaguchi et al.[7] analyzed the structure of the γ-Glu-Val-Gly (EVG) and CaSR complex using cryo-electron microscopy, and Yamamoto et al.[8] discovered that the GPRC6A receptor is a key target for ornithine in inducing the taste of kokumi, revealing the neural pathways through which it enhances the perception of umami and saltiness. However, this mechanism was only verified in animal models and did not include human sensory feedback. Yang et al.[9] combined with peptidomics to verify its flavor activity, revealed that kokumi peptides in chicken soup enhance flavor persistence and mouthfulness through activation of the CaSR

pathway. This provides a scientific basis for the development of traditional stock flavors. Yi et al.[10] and Dellafiora et al.[11] revealed the mechanism of action of γ-glutamyl tripeptide using computer simulation technology. By integrating food chemistry, neurobiology, computational chemistry, and artificial intelligence, a model describing the relationship among "structure-activity-perception" is constructed to advance sensory science theory and facilitate interdisciplinary integration. Han et al.[12] established the kokumi peptide sequence library and structure-activity relationship map, and used machine learning to predict new peptides. This approach facilitates the precise design of functional food components and enhances the development of the functional peptide database.

The national "Healthy China 2030" Planning Outline emphasizes improving the nutritional and flavor quality of food while promoting the development of green and healthy condiments. In this context, in-depth research on kokumi peptides not only helps to uncover the molecular basis of food flavor formation, but also provides essential technical support for the development of new healthy foods that are low in salt, low in fat, and rich in flavor. Li et al.[13] reviewed the latest progress on kokumi peptides in salt reduction. Kokumi peptides can partially replace salt in meat products and condiments, supporting the implementation of the national "Three Reductions and Three Health" policy and achieving the goal of reducing salt without compromising flavor. In daily life, functional peptides can be extracted from agricultural by-products such as yeast extract, mushroom scraps, and hemoglobin hydrolysate. Additionally, kokumi peptides have been extracted from underutilized protein sources, such as the skin of dry-cured lamb, to develop high-value-added products, thereby enhancing resource utilization[4,6,14]. Five peptide segments (FTKK, CKEVVRNANE, EELNVPG, VPNSAEER, and YPVEPFTER) exhibiting kokumi activity were isolated and purified from the by-products of enzyme-modified butter in a previous study. This indicates that butter by-products can serve as substrates for producing taste-active peptides, thereby creating added value and new application opportunities, making the approach more environmentally friendly[15].

2 The main sources of kokumi peptides and food substrates

The sources of kokumi peptides are primarily categorized into three groups: plant-based, animal-based, and microbial. Among plant-based sources, soybeans and their fermented products—such as soy sauce, douchi, and natto—are rich in γ-glutamyl peptides, which are catalyzed by the microbial enzyme γ-glutamyl transpeptidase (γ-GTP)[16]. Studies have found that peptides such as γ-Glu-Cys-Met and γ-Glu-Cys-Phe, extracted, separated, and purified from doenjang soup, exhibit a stronger umami-kokumi synergistic effect than individual components when combined with monosodium glutamate (MSG) or nucleotides. However, these samples were limited to local Korean consumers[17–19]. Hayashi et al.[20] developed a salt-free process to extract kokumi substances from plant protein hydrolysates, thereby avoiding the interference of sodium ions with flavor characteristics. Additionally, edible fungi are a rich source of kokumi peptides, containing abundant proteins and free amino acids. Feng et al.[21] gradually isolated and purified multiple candidate peptides from the hydrolysate of *Agaricus bisporus* using ultrafiltration (UF), gel filtration chromatography (GFC), and reversed phase-high performance liquid chromatography. These peptides enhanced the thick mouthfeel through CaSR activation, and a "sensory-computational" dual validation model confirmed their significant "long-lasting aftertaste" effect. Yeast extract is an important raw material in the modern condiment industry, rich in nucleic acid degradation products (disodium 5′-inosinate and disodium 5′-guanylate) and various small peptides[22]. Lao et al.[23] identified a novel kokumi peptide, γ-Glu-Glu-Tyr, from yeast extract and revealed its interaction with the dual targets taste receptor type 1 member 1 (T1R1)/T1R3 and CaSR through computer simulation technology.

Animal-derived kokumi peptides are primarily sourced from four major categories: pork, chicken, dairy, and aquatic products. Li et al.[24] use of bacterial γ-glutamyl transferase (GGT) to increase the γ-Glu peptide content in porcine hemoglobin hydrolysate holds significant potential for salt reduction. Adding 0.1% (*m*/*m*) of this peptide can replace 30% (*m*/*m*) of NaCl. However, the bitter taste generated during enzymatic hydrolysis necessitates additional debittering treatment. Similarly, peptides produced through lactic acid bacteria fermentation exhibit the strongest activity at pH 5.5[25–26], but the bitterness resulting from fermented whey protein also requires mitigation. Kokumi peptides have been identified in Spanish dry-cured hams, enabling a reduction of the salt content by 30% (*m*/*m*) while still preserving kokumi activity. However, the resulting increase in water activity may negatively impact the shelf life of the products[27–28]. Research has found that after 18 months of maturation, the kokumi peptide content of hard cheese exceeded 200 mg/kg, which is positively correlated with the intensity of the "umami aftertaste"[29–30]. The results indicate that the difference in the concentration of EVG in cheese made from milk and goat milk is due to differences in the amino acid sequences of casein[31]. However, the peptide content in cheese made from different milk sources varies significantly, making standardized production challenging. A variety of hydrophobic dipeptides with similar properties have also been found in fish protein hydrolysates[32]. Ramesh et al.[33] reported that marine animal by-products, such as fish skin gelatin and shellfish cooking liquid, can increase the yield of γ-glutamyl peptides through targeted enzymatic hydrolysis. Additionally, Yamamoto et al.[2] noted that adding kokumi peptides to cell-cultured meat can effectively simulate the "aftertaste" of traditional meat. In the future, it is essential to deepen the understanding of peptide stability regulation and receptor dynamic response models, in order to promote the widespread application of animal-derived kokumi peptides in food development.

3 Current research progress

Research on kokumi peptides started earlier abroad, and the field is now well-established. They first discovered that EVG has typical kokumi activity and confirmed that it exerts its effect by activating the human CaSR. Yamamoto et al.[34] further clarified the influence of the number of glutamate residues and the N-terminal structure on CaSR activation ability, proposing that the "$[Glu]_3$" domain is a key feature of highly efficient agonists. Regarding detection and identification techniques, Kim et al.[35] developed an liquid chromatography-tandem mass spectrometry (LC-MS/MS) quantitative method and successfully determined the contents of various γ-glutamyl peptides in traditional Korean fermented soy sauce (ganjang) and doenjang. The Hofmann team discovered that acetylene-oxidized lipids generated during the heat treatment of

avocados have a kokumi-enhancing effect, thereby expanding the research boundaries of non-peptide kokumi substances[36]. Subsequently, Phewpan et al.[37] identified multiple kokumi substances in Pla-ra, a fermented freshwater fish product from Thailand, and linked them to specific lactic acid bacteria communities. Laffitte et al.[38] were the first to demonstrate that domestic cats (*Felis catus*) can also perceive the taste of kokumi, indicating that this sensory pathway is somewhat conserved across mammals and advancing cross-species research.

Although research on kokumi peptides in China began relatively late, it has developed rapidly in recent years. With the transformation and upgrading of the food industry and the increasing consumer demand for natural flavor substances, domestic scholars have actively engaged in this field and achieved a series of significant results. They systematically studied the flavor mechanism of kokumi peptides in yeast extract and combined molecular docking with molecular dynamics simulation to reveal their binding mode with the CaSR[39]. Tu et al.[5] synthesized a novel umami-salty-kokumi synergistic enhancement peptide using a bitter dipeptide derived from the proteolytic hydrolysate of defatted peanuts. Previous studies have shown that utilized bacterial GGT to catalyze the generation of γ-glutamyl peptides from porcine hemoglobin hydrolysate, significantly enhancing the flavor stability and kokumi intensity of sausages[24,40]. Shibata et al.[41–42] found that heat treatment of soybean seeds can increase the dissolution rate of kokumi components, providing a foundation for optimizing soy milk quality. In recent years, collaboration between China and foreign countries has become increasingly close. For example, Tu et al.[5] and Yang et al.[43] have jointly published several high-impact papers, demonstrating that Chinese scholars are rapidly integrating into the mainstream of international kokumi research. Both domestic and international studies have recently focused on breakthroughs at the mechanistic level, particularly making significant progress in understanding the receptor recognition mechanism.

4 The separation, purification, identification and synthesis technology of kokumi peptides

The effective separation and purification of kokumi peptides is crucial for in-depth research on their properties and applications. At present, a variety of separation techniques are widely applied to obtain kokumi peptides from complex biological systems or food matrices. It can be seen from Figure 1, the methods for separation, purification, and identification are similar to those of other taste peptides. The following are several commonly used methods for separation and purification. Firstly, the kokumi peptides extracted from various sources are preliminarily separated by UF. UF is a membrane separation technology that separates molecules based on their size. It utilizes UF membranes of different pore sizes to selectively retain or allow molecules of specific sizes based on the molecular weight range of kokumi peptides. According to Liang et al.[44], oligopeptides with molecular weights below 1 000 Da are the main flavoring agents in yeast extracts, chicken soup and Jinhua ham. Rhyu et al.[45] also discovered that peptides with molecular weights between 500 and 1 000 Da have the strongest flavor effect when studying soybean paste. In *A. bisporus*, Feng et al.[21] found peptides with molecular weights ranging from 200 to 1 000 Da have the strongest thickness flavor. Kokumi peptides can be found in many different foods, including by-products of enzyme-modified butter[15] and yeast extract[23]. Many undeveloped kokumi peptides still need further exploration and discovery. Next, GFC, also known as molecular exclusion chromatography, is adopted, which is a method of separation based on the size and shape of molecules. It uses gel media with different pore sizes. When the sample solution passes through the gel column, smaller molecules can enter the pores of the gel particles, thus remaining in the column for a longer time. However, larger molecules cannot enter the pores and directly flow out of the column through the gaps between the gel particles. In this way, kokumi peptides can be separated from other

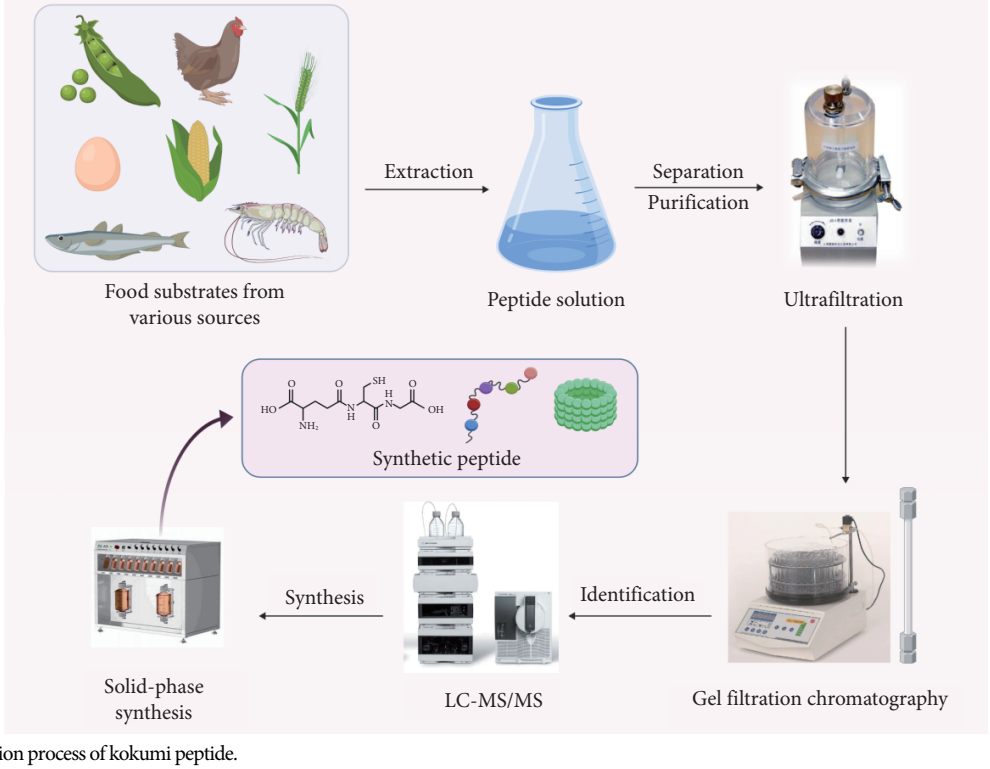

Figure 1 The extraction process of kokumi peptide.

impurities according to their molecular size. GFC has mild separation conditions, does not damage the structure and activity of peptides, and can obtain relatively pure kokumi peptide components. Some studies have adopted ion-exchange chromatography to precisely separate peptides based on their isoelectric points and charge densities, thereby enhancing the purity of kokumi peptides. Additionally, affinity chromatography, with its high selectivity, can efficiently separate the target kokumi peptides.

Accurate identification of the structure of kokumi peptides is key to understanding its flavor mechanism and applications. Common methods include LC-MS/MS (by separating components, obtaining molecular weight through primary mass spectrometry, obtaining sequence information through secondary MS, and comparing the database to determine the structure, high-resolution mass spectrometry can precisely quantify and discover new peptides)[46–47], nuclear magnetic resonance (providing three-dimensional structure and dynamic information), it is close to the physiological environment but requires high purity and is time-consuming. Infrared spectroscopy and Raman spectroscopy (rapid and non-destructive analysis of chemical bond vibrations for preliminary identification or in combination with other techniques), as well as amino acid composition analysis (providing information on amino acid types and contents, laying the foundation for structural determination and assisting in determining whether it belongs to a new type of peptide). Based on the understanding of its structure and mechanism, the methods for synthesizing kokumi peptides to meet the demand for large-scale production include solid-phase synthesis, liquid-phase synthesis (suitable for short peptides, with mild conditions and high yield), and genetic engineering (synthesis in host cells through expression vectors). After mastering the synthetic method, large-scale production is possible, but important technical challenges such as optimizing expression efficiency and purification need to be addressed.

5 The receptor selection mechanism of kokumi peptide

In recent years, kokumi peptides have been identified in many foods. Numerous studies, regardless of the source, have primarily used the CaSR as the receptor for kokumi peptides. However, there remains considerable debate about the existence of other auxiliary receptors. Previous research has demonstrated that kokumi peptides can activate the CaSR, thereby enhancing flavor richness[48–50]. CaSR is located on the membrane surface of taste cells and belongs to the class C family of G protein-coupled receptors, comprising 1 078 amino acid residues[51–52]. As early as 2009, Gabriel et al.[52] identified the presence of CaSR in mouse taste cells and confirmed that CaSR is a potential taste receptor responsible for eliciting strong taste sensations in mammals. Since then, numerous researchers have investigated the relationship between thick taste perception and CaSR.

Kuroda et al.[50] found that the intensity of the thick taste of substances such as glutathione (GSH, γ-Glu-Cys-Gly), γ-Glu-Ala, γ-Glu-Val, γ-Glu-Cys, γ-Glu-Abu-Gly (Abu: α-aminobutyric acid), and EVG was significantly positively correlated with CaSR activity. This study also demonstrated that when the CaSR-specific antagonist NPS-2143 was added, the taste intensity of GSH and EVG was significantly reduced, indicating that CaSR is involved in the taste effect of kokumi substances[53]. The research by Maruyama et al.[49] further confirmed this conclusion and found that CaSR does not directly participate in the signal transduction of umami or sweetness, proving that taste cells expressing CaSR constitute a distinct subpopulation from T1R3-expressing cells, which are responsible for umami and sweetness receptors, and that CaSR-expressing cells are the primary detectors of kokumi substances. This further confirms the role of CaSR as a core receptor, clarifies the involvement of the T1R3 subunit in the umami-rich flavor synergistic effect, and elucidates the corresponding role of mGluR1 in tongue epithelial cells in response to long-chain γ-Glu peptides.

Additionally, the amino acid sequence of γ-glutamyl peptides with strong flavor activity may influence the activation of CaSR. Amino et al.[54] found through studying the relationship between the structure of γ-glutamyl peptides with strong flavor activity and CaSR activity that γ-glutamyl dipeptides or tripeptides with *L*-configuration at the N-terminus, as well as medium-sized aliphatic neutral substituents attached to the second residue, have a strong ability to activate CaSR. To better understand the characteristics of kokumi peptides, this study summarizes the sequences of kokumi peptides from various sources reported in the literature, along with the selected receptor structures, as shown in Table 1.

However, due to the complex and diverse arrangements of amino acids, a single sequence pattern cannot represent the amino acid sequence characteristics of γ-glutamyl peptides with strong flavor activity. Therefore, the mechanism underlying the rich flavor of kokumi peptides requires further investigation. Everyone tends

Table 1 The sources, sequences, and receptors of kokumi peptides.

Source	Peptides	Receptor	Reference
Lactones	δ-Decalactone	CaSR	[55]
GSH	GSH-sugar Maillard reaction products (MRPs)	CaSR	[56]
Boletus edulis	Tyr-Arg-Phe	CaSR	[57]
Fermented doenjang	γ-Glu-Phe, EVG		[58]
Synthetic sample	Ile-Leu, Ile-Ile, Leu-Leu, Leu-Ile	CaSR	[59]
Sparkling wines	Ala-Lys, Asp-Leu, Asp-Ile, Asp-Val, Glu-Glu, Asp-Gly-Val, Asp-Gly-Leu, Asp-Gly-Ile, Asp-Asn-Val, Glu-Val-Gly	CaSR	[60]
Bacillus species	γ-Glutamyl peptides		[61]
Enzymatic synthesis	*N*-Lauroyl phenylalanine, γ-glutamyl peptides	CaSR	[62–64]
Chicken broth	ELKDHLLH, ELQKLH, LETHFD, TPDFVR, TPELLH		[65]
Rat taste cells	GSH and EVG	CaSR	[66–67]
Shijimi clams	*L*-Ornithine	GPRC6A	[8]

to use CaSR, however, a new receptor GPRC6A has recently been discovered, providing additional options for receptor use and expanding the scope of research.

6 The enzymatic synthesis mechanism of GGT

In recent years, research on kokumi peptides has primarily focused on γ-glutamyl peptides, suggesting that all peptides containing this structure exhibit kokumi activity. Chang et al.[39] extracted and screened 10 polypeptides from yeast extract. All kokumi peptides were able to bind to the CaSR to form complexes. Among them, GSH, γ-Glu-Leu, and γ-Glu-Tyr exhibited the highest binding affinities. γ-Glutamyl peptides are mainly synthesized through enzymatic catalysis. The proteases reported in the literature with the ability to synthesize γ-glutamyl peptides mainly include three types: GGT (EC 2.3.2.2), glutaminase (EC 3.5.1.2), and glutamyl cysteine synthase (EC 6.3.2.2). Most studies have concentrated on synthesis via the GGT enzyme[68]. Here, the enzymatic synthesis mechanism of GGT is summarized and explained.

As depicted in Figure 2, the catalytic reaction of GGT involves the transfer of γ-glutamyl groups from donor substrates to acceptor substrates[69–70]. This catalytic process occurs in two steps. The first step, known as acylation, involves the γ-position oxygen atom of the N-terminal threonine residue in the GGT small subunit attacking the carbonyl carbon of the γ-glutamyl compound. This attack cleaves the γ-glutamyl bond present in compounds such as GSH and glutamine. Subsequently, the γ-glutamyl moiety from the donor substrate forms a covalent bond with the enzyme, creating a γ-glutamyl-enzyme intermediate. The second step, deacylation, involves the use of γ-glutamyl acceptor as a nucleophile to enter the active site and attack the carbonyl group of the intermediate. This reaction breaks the bond, generating a new γ-glutamyl compound and releasing the enzyme. Experimental results indicate that the second step, specifically the hydrolysis of the intermediate, proceeds more slowly than the first step and constitutes the rate-limiting step of the catalytic reaction[71].

Depending on the receptor, GGT can catalyze three types of reactions: 1) transpeptidation reaction: when amino acids and short peptides act as γ-glutamyl receptors, GGT catalyzes the transfer of the γ-glutamyl group from γ-glutamyl compounds such as GSH to form new γ-glutamyl compounds. 2) Hydrolysis reaction: when water molecules act as γ-glutamyl receptors, GGT catalyzes the transfer of γ-glutamyl groups from donor substrate molecules to water, forming glutamic acid. 3) Autotranspeptidation reaction: γ-glutamyl compounds act as both donors and acceptors of γ-glutamyl groups.

As GGT is a transferase rather than a synthase, it does not consume energy compounds such as ATP and exhibits high enzymatic activity. Therefore, this method holds promising potential for the large-scale production of γ-glutamyl peptides in the food industry. In 2017, they demonstrated a novel use for a commercially available glutaminase, which can function as a γ-glutamyltranspeptidase in kokumi seasoning production. γ-Glutamylated gluten hydrolysate was the most preferred sample, significantly enhancing thickness, kokumi, and umami, with a moderate increase in saltiness[72]. And these peptides, including [γ-Glu]$_{(n\leq5)}$-Phe/Val/Met/Tyr/Leu/His/Tau, γ-glutamyl-*S*-allyl-cysteine and γ-glutamyl-*S*-methyl-cysteine, have been successfully

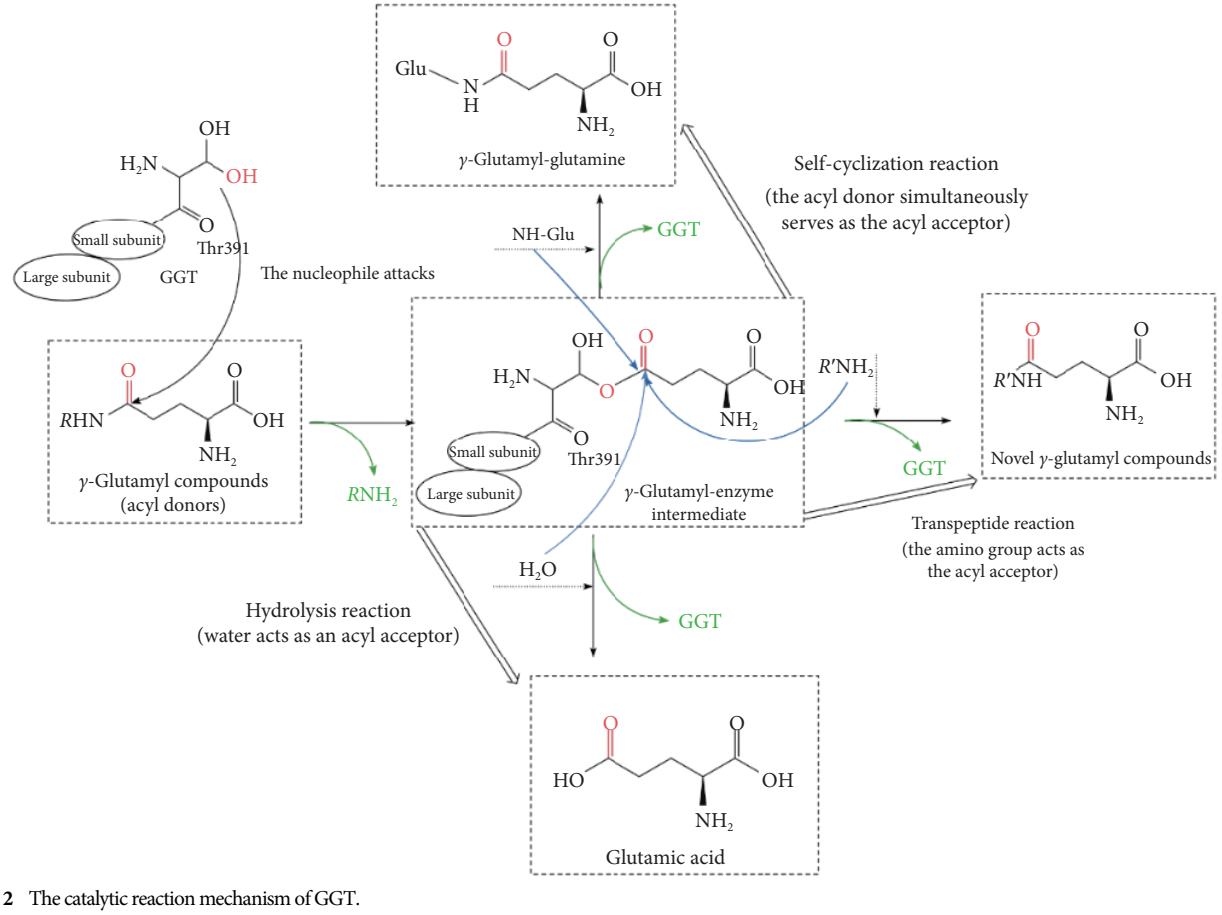

Figure 2 The catalytic reaction mechanism of GGT.

synthesized via catalysis with γ-glutamyl transpeptidase and *L*-glutaminases[73]. However, the food industry currently lacks food-grade γ-GTP that is both highly specific and highly active and suggested the rising difficulty for synthesis when the number of donors increases in the reaction mixtures[74]. Employing synthetic biology techniques to efficiently express safe GGT is a key developmental direction for producing food-grade γ-glutamyl peptides.

7 The flavoring mechanism of kokumi peptides

Human perception of taste begins in the mouth. Research shows that taste receptors are located in the taste buds, which are the peripheral organs of taste. Taste buds consist of clusters of columnar sensory cells situated on the tongue. Molecular recognition of taste substances occurs within the taste receptor cells at the apex of the taste buds. The receptors in these cells confer specificity to taste stimuli, which are then transmitted via taste nerves and ultimately processed by the taste center in the brain[75].

As shown in Figure 3, peptides and amino acids are essential compounds for kokumi sensing in the kokumi-enriched components[76]. After being recognized by taste receptor cells, small molecules and biomarkers such as proteins or peptides are transported to specific CaSR. This interaction induces conformational changes in G proteins, generating action potentials and modulating signal transduction, which affects the timing, strength, and sensitivity of taste perception by lowering the minimum taste threshold recognized by the human body. Subsequently, neurotransmitters are released. The signals produced by these proteins, peptides, and small molecules are processed by the taste center of the brain, completing the entire taste perception process.

Under normal circumstances, adults have over 9 000 taste buds, while infants may have more than 10 000. Each taste bud is usually composed of about 50–100 slender taste cells. A classification scheme that integrates ultrastructural features and patterns of gene expression with cell function generally recognizes three cell types (in order of their relative abundance): type I, type II and type III cells[78]. Type I cells (glial-like cells) are responsible for absorbing, degrading, and clearing neurotransmitters. Type II cells (receptor cells) express G protein-coupled receptors that bind to bitter, sweet, and umami substances. They also contain downstream signaling components such as phospholipase C β2 (PLCβ2) and G proteins[79]. Type III cells (presynaptic cells) form synaptic connections with nerve endings and receive and integrate signals from type II cells. Previous studies have detected the expression of the CaSR in type III taste cells at both the mRNA and protein levels, with CaSR distributed throughout the cell membrane[52,80]. However, considerable controversy remains regarding whether CaSR is also expressed in type I and type II cells. Bystrova et al.[80] employed serial multistandard-assisted reverse transcriptase-polymerase chain reaction technology to demonstrate that CaSR mRNA is expressed in some type I cells at the single-cell level, but it was not detected in type II cells. However, they did not establish the functional coupling between CaSR and PLC-dependent Ca^{2+} signaling pathways in type I cells. Therefore, CaSR may transmit signals in type I cells through a non-PLC-dependent mechanism. Conversely, Gabriel et al.[52] used immunofluorescence to show that CaSR is associated with type II (PLCβ2) and type III cells neural cell adhesion molecule co-expression. The research also conducted by Maruyama et al.[49] demonstrated that EVG can induce PLC-dependent Ca^{2+} release, thereby increasing the intracellular Ca^{2+} concentration. This cascade reaction occurs independently of the presence of extracellular Ca^{2+}.

As indicated in Figure 4, this figure provides a detailed description of the role of CaSR in taste cells and the resulting changes in signaling pathways[81]. In type I cells, the activation of CaSR at the basal apex may be related to the regulation of the potassium cycle by the apical renal outer medullary potassium (ROMK). This channel may be involved in the recovery of K^+ accumulated in the narrow space between type II and type III cells[82]. CaSR is most likely to function in type II cells, causing the transduction of a kokumi flavor. In type II cells, when CaSR homodimers or possibly heterodimers are activated, the PLCβ2 can be initiated. PLCβ2 catalyzes the formation of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol 4,5-diphosphate (PIP_2), which prompts the endoplasmic reticulum (ER) to release Ca^{2+} through the IP_3 receptor (IP_3R). The increase of Ca^{2+} within cells leads to the depolarisation of the cell through the actions of the Na^+ channel transient receptor potential cation channel, subfamily M, member 5 (Trpm5), delayed

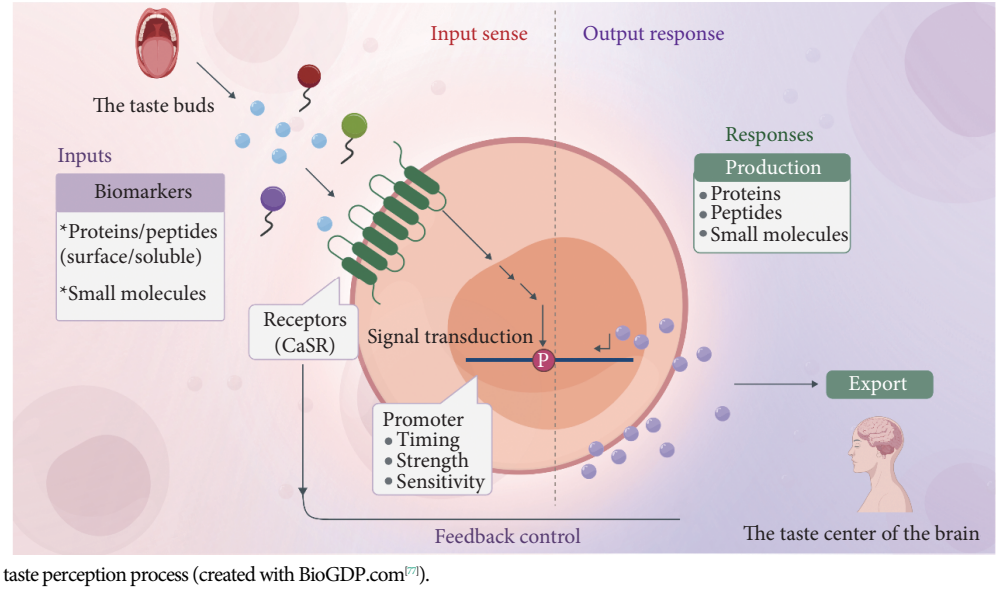

Figure 3 The entire taste perception process (created with BioGDP.com[77]).

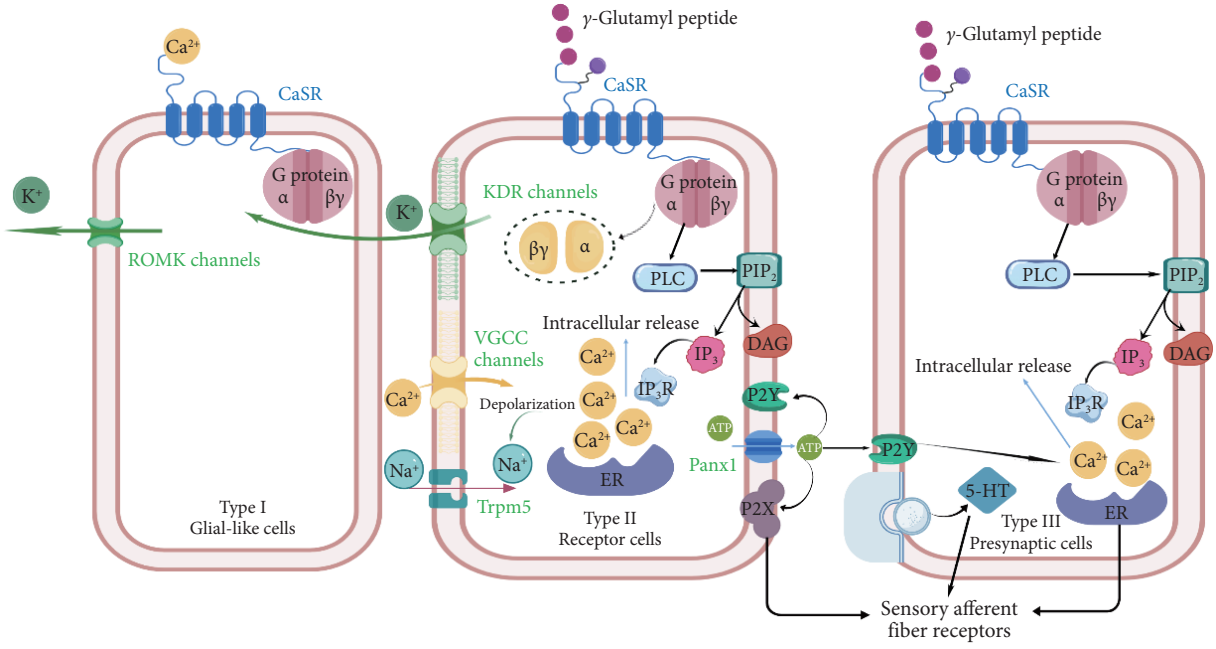

Figure 4 The signaling pathways mechanism of CaSR in taste cells.

rectification potassium channels (KDR), and voltage-gated calcium channels (VGCC). Subsequently, the cells release ATP through pannexin 1 (Panx1), activating the P2Y and P2X receptors on sensory nerve fibers[83]. In type III presynaptic cells, γ-glutamyl peptide activates apical CaSR, leading to an increase in intracellular Ca^{2+} concentration through PLC dependence, with a reaction pathway similar to that of type II cells. The increase in intracellular Ca^{2+} concentration in type III cells is associated with the release of serotonin 5-hydroxytryptamine (5-HT), which can inhibit type II receptor cells. However, it is still unclear whether occurs in a way that depends on case.

When type III taste cells expressing CaSR are exposed to *L*-Phe, *L*-Arg, and γ-glutamyl peptides (such as GSH and EVG), intracellular transient Ca^{2+} changes are induced, and this response can be blocked by non-specific PLC inhibitors[49,80]. Furthermore, high concentrations (3 μmol/L) of negative allosteric modulators can also inhibit the Ca^{2+} response induced by EVG, indicating that the action of these peptides depends on the activation of CaSR[49]. Therefore, CaSR is regarded as one of the key receptors of kokumi's taste. Its activation can enhance the perceived thickness and strength of other basic tastes, especially umami, thereby improving the overall flavor quality. More and more evidence indicates that CaSR may influence the body's appetite preference for nutrients by regulating taste perception.

8 Application in industry

Kokumi peptides generally do not exhibit a distinct taste when added to pure water. However, when incorporated into solutions or food media containing basic taste substances, they can enhance the original taste intensity and simultaneously improve the texture. As illustrated in Figure 5, kokumi peptides not only enhance umami but also increase saltiness and sweetness, thereby improving the thickness, continuity, and mouthfulness of food[84–87]. They thereby constructed a novel hypothetical receptor, T1R3-MSG complex. The umami-enhancing effect of typical kokumi-active γ-glutamyl peptides was verified by sensory evaluation[88].

Kuroda[89] discovered through CaSR detection and sensory evaluation screening that EVG is a potent umami-enhancing peptide. Descriptive analysis revealed that chicken soup containing EVG exhibited significantly greater consistency, richness, and

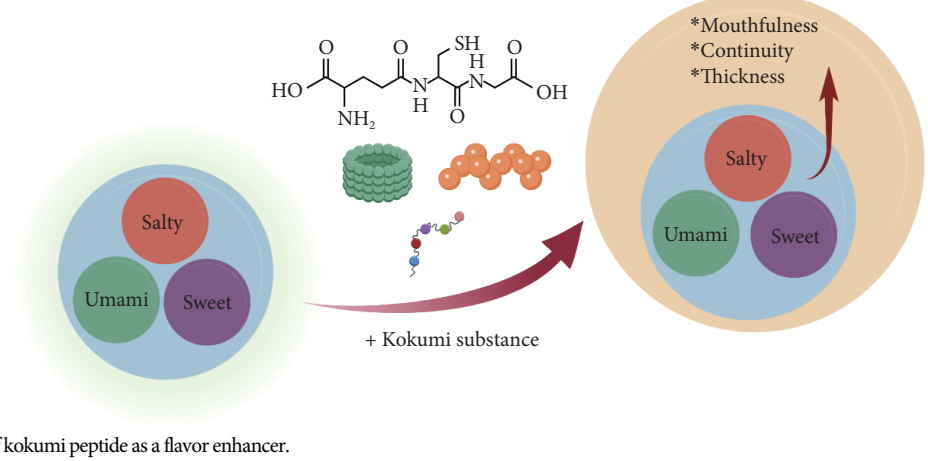

Figure 5 The process of kokumi peptide as a flavor enhancer.

persistence compared to the control group. Additionally, EVG significantly enhances the greasiness, richness, and aftertaste of low-fat peanut butter, and markedly improves the taste consistency of low-fat cream pudding. As an umami enhancer, EVG itself is tasteless, making it suitable for improving the flavor of both salty and sweet foods, including low-fat products.

MRPs were prepared using pea protein as the raw material through enzymatic hydrolysis, followed by a thermal reaction with reducing sugar. When the MRPs content was 0.7% (*m*/*m*) and the NaCl content was 0.4% (*m*/*m*), the saltiness of the solution was higher than that of the control group containing 0.5% (*m*/*m*) NaCl. This indicates that even with a 20% (*m*/*m*) reduction in NaCl content, the perceived salinity was not diminished. These results suggest that these peptides have promising potential as effective chicken flavor enhancers in the food industry[90–92]. Additionally, research shows that adding kokumi peptide, isolated and purified from *A. bisporus* and bovine bone marrow extracts, to model chicken soup enhances its complexity, mouthfulness, and palatability[93–94].

9 Challenges and future development directions

The sources of kokumi peptides are very extensive. Currently, through separation and purification, as well as the combination of sensory and electronic tongue analysis techniques, its application potential in reducing salt, enhancing umami, saltiness, and sweetness in food media, improving food taste, and developing functional products can be verified. However, during its development process, it lacks a unified sensory evaluation standard. The inconsistent sensory groups and scoring scales used by different research teams, as well as the inconsistent standard substances or their corresponding concentrations selected, make it difficult to make horizontal comparisons of the results. The functional verification methods have limitations. Most studies only rely on *in vitro* cell models and computer simulation techniques for evaluation, lacking the support of animal or human trials. The structure-activity relationship is not clear. Which amino acid residues determine significant differences in kokumi activity between each source, and is there a systematic rule on how side chain modification affects taste? Industrialization is rather difficult. The cost of natural extraction is high, the extraction rate is low, chemical synthesis is complex, and enzymatic preparation has not yet been scaled up. γ-Glutamyl peptide is easily degraded by peptidase and may become inactive during processing. Therefore, it is necessary to develop a stabilized encapsulation technology and it is worth noting that current sensory evaluations of kokumi peptides are largely region-specific. Future studies should prioritize cross-cultural sensory analyses to validate the global applicability of these regional findings.

In future research and development, it is necessary to construct a Chinese kokumi peptide database, integrate the reported active peptide sequences, sources, structures, and activity data, and establish an open and shared information platform. Develop an intelligent prediction model dedicated to kokumi peptides, and train the kokumi peptide recognition model using machine learning algorithms to achieve rapid virtual screening. Promote multi-omics joint analysis, integrating proteomics, metabolomics, and flavomics, to comprehensively analyze the dynamic changes of kokumi peptides during food processing. Strengthen functional verification research, conduct animal experiments or small-scale human trials, and evaluate its long-term intake safety and flavor enhancement effect. Promote collaborative innovation among industry, academia, and research institutions, encourage cooperation between universities, research institutes, and condiment enterprises, and accelerate the transformation and application of experimental results. Clarify the naming rules, testing methods, and label identification norms for kokumi substances, further promote the formulation of industry standards, and facilitate the healthy development of the industry.

Kokumi peptide, as a new generation of natural flavor enhancer, also plays a significant role in the field of clean labels and is currently at a critical stage of transitioning from basic research to industrialization. Internationally, we have gained a leading edge in receptor mechanisms, structural analysis, and consumer science, while domestically, with abundant food resources and rapidly developing omics technologies, we are catching up. In the future, efforts should be made to further enhance the connection between basic research and industrial demands, smooth out the entire chain from "discovery to mechanism, preparation to application", and promote independent innovation and development in the field of functional flavor substances in China.

Conflict of interest

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.

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Author contributions

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