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Umami and saltiness enhancements of textured pea proteins by combining protease- and glutaminase-catalyzed reactions

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ABSTRACT

Plant-based meat analogs (PBMAs) have attracted attention owing to their various advantages, however, their taste limits their application, requiring improvement of the umami and saltiness levels while meeting clean-label requirements. Enzymatic treatments for food processing are effective strategies for developing clean-label food products because enzymes are not considered food additives. In this study, we aimed to enhance the umami and saltiness intensity of PBMA patties by combining protease- and glutaminase-catalyzed reactions. For the production of extrudates to construct PBMA patties, enzymatically hydrolyzed pea proteins (eHPP) were produced via enzyme catalysis combinations, followed by the preparation of eHPP-mixed textured pea protein (eTPP) from eHPP and starch. Sensory evaluation revealed that the umami, kokumi, and saltiness levels of the eTPP-based patties containing 0.5% NaCl were significantly higher than those of the control patties containing 0.5% NaCl. Notably, the eTPP-based patties exhibited a 20% salt reduction. By screening for saltiness-enhancing amino acids and peptides released from eTPP-based patties in artificial saliva, the combination of Glu, Arg, Lys, and the separated peptide 3 was determined important in enhancing the saltiness intensity of NaCl. Moreover, it was revealed that the saltiness-enhancing peptide 3 may be a Maillard-induced peptide, based on the Lys residues in Glu-Gly-Lys-Gly and 5-hydroxymethylfurfural condensed from Glucose in starch during the extrusion process. Our findings suggest that the combination of proteases and glutaminases could be an attractive approach to enhance the umami and saltiness levels of PBMA products while meeting clean-label requirements.

1. Introduction

The global population was 7.5 billion in 2017 and is expected to reach 8.5 and 10 billion by 2030 and 2050, respectively (UN, 2024). With this exponential growth, the "protein crisis," in which the supply-demand balance of animal-derived protein sources collapses, will become a challenge as early as 2030 (McClements and Grossmann, 2022). To address this, plant-based meat analog (PBMA) products are attracting attention as a promising option (He et al., 2020). Currently, soy-, pea-, and wheat-derived proteins are the three main proteins used in PBMA products (Plattner et al., 2024; Sakai, 2024). Among these, research on PBMA products is rapidly shifting towards pea proteins, because of the increase in demand for clean-label ingredients (Plattner et al., 2024). Only pea ingredients can meet the clean-label requirements, because they are non-genetically modified and are considered lower allergen crops (Fischer et al., 2020; Plattner et al., 2024). Moreover, pea protein exhibits processing advantages for the development of PBMA products, including suitable solubility, gelling, emulsifying, and liquid-holding abilities (Kumar et al., 2022; McClements and Grossmann, 2022). Thus, food and research communities focus on developing pea-based PBMA products to meet the clean-label requirements.

Despite many technological innovations, the palatability of commercially available PBMA products is yet to meet the consumer standards established by animal-based meat (McClements and Grossmann, 2022). In particular, pulse-derived proteins have a light taste or beany flavor, which is quite different from that of animal proteins (McClements and Grossmann, 2021). To reduce any off-flavors from PBMA products, low-moisture extrudates are usually washed with water multiple times (He et al., 2020). However, this process is not able to completely remove off-flavors remaining in PBMA products; furthermore, it poses challenges in terms of manufacturing, washing time, and costs (McClements and Grossmann, 2022). Therefore, flavor enhancers are essential food additives for creating a satisfactory culinary

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experience for PBMA products (McClements and Grossmann, 2022). Typical flavor enhancers include amino acids, yeast extracts, vegetable powders, and salt, which are able to overcome potential blandness and mask off-flavors, thereby contributing to depth, complexity, and overall sensory appeal (Jang and Lee, 2024; Bakhsh et al., 2021). In particular, glutamate and yeast extracts are used to enhance the umami or kokumi taste, while salt is used for saltiness (Tomé, 2021; Yamamoto and Inui-Yamamoto, 2023). Although the addition of these flavor enhancers is an effective strategy, the increasing consumers' demand for clean-label products requires the reduction of the amounts or varieties of food additives (Asioli et al., 2017). Similarly, the increasing consumers' concern about diseases associated with salt over intake requires salt reduction using saltiness enhancement compounds (Cook et al., 2016; Chan et al., 2016). To overcome these challenges, the scientific community and food industry focus on the development of more acceptable strategies for umami and saltiness enhancement while meeting clean-label requirements.

Recently, enzymatic treatments for food processing and ingredient production have attracted significant attention as effective strategies for producing clean-label foods, because denatured or inactivated enzymes are not considered food additives (Asioli et al., 2017; Sakai et al., 2024c; Dai and Tyl, 2021; Scarton et al., 2021). Thus, taste modification using enzymes could be an attractive approach to increase the umami or saltiness levels of PBMA products while reducing the amount or variety of additives. The enzymes synthesizing amino acids and peptides with umami taste include glutamic acid synthesized by glutaminase (EC 3.5.1.2) and γ-glutamyl peptides synthesized by γ-glutamyl-transpeptidase (EC 2.3.2.2) (Zhang et al., 2019). Furthermore, the enzymes that synthesize amino acids and peptides with saltiness enhancements include protein hydrolysates synthesized by proteases (EC 3.4.-.-) or γ-glutamyl peptides synthesized by γ-glutamyl-transpeptidase (Le et al., 2022). However, the ability of enzymes and their related products to enhance the umami or saltiness levels of food remains understudied.

In this study, we aimed to develop a production process for pea-based PBMA products to improve their umami and saltiness levels while meeting clean-label requirements. Three enzymes were used: bacterial protease, fungal protease, and glutaminase. Bacterial proteases primarily possess *endo*-peptidase activity and degrade proteins into larger peptides. In contrast, fungal proteases mainly possess *exo*-peptidase activity, which degrades larger peptides into smaller peptides and amino acids (Song et al., 2023). Glutaminase catalyzes the deamidation of free Gln to Glu, which imparts an umami taste (Martínez et al., 2022). We first produced enzymatically hydrolyzed pea proteins (eHPP) using three enzyme combinations and manufactured an eHPP-mixed textured pea protein (eTPP). The umami and saltiness levels of the eTPP-based patties were investigated, and the salt reduction rate was evaluated using sensory tests and electronic tongue analysis. Subsequently, we analyzed the amino acids and peptides released from the eTPP-based patties in artificial saliva and screened their saltiness-enhancing components. Moreover, we measured the volatilization amounts of beany off-flavor from eTPP-based patties and investigated the relationship between the amounts of off-flavor compounds and the hydrophobic degrees of the hydrolyzed proteins at each production process. Then, the effects of the wash process on the physical and sensory properties of the PBMA products were examined. Finally, we characterized the *in vitro* digestibility of eTPP-based patties using the INFOGEST method.

2. Materials and methods

2.1. Materials

Pea protein isolates (PPI) and potato starch were obtained from PURIS Foods Inc. (Minneapolis, MN, USA) and Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan), respectively. Food-grade bacterial and fungal proteases and bacterial glutaminase (Amano Enzyme Inc., Nagoya, Japan) were commercially available products (Thermoase PC10FA, Protease "Amano" UF, and Glutaminase SD-C100SNA).

2.2. Preparation of textured vegetable proteins

eTPP was prepared as follows (Fig. 1A): Enzyme assays were performed in mixtures containing 10% PPI and 3000 U/g-protein fungal protease, 1000 U/g-protein bacterial protease, and 1 U/g-protein glutaminase. The enzymatic reactions were performed at 50 °C for 1 h. After deactivation at 90 °C for 10 min, the reaction solution was powdered using a spray dryer (Model T-20, Henningsen Nederland B.V., Schouwslootweg) with inlet and outlet temperatures of 140 and 90 °C, respectively. The obtained eHPP was extruded using a twin-screw extruder (TX-57, Wenger Manufacturing LLC, Kansas, USA). The control TPP was composed of 85% PPI and 15% starch, while eTPP was composed of 70% PPI, 15% eHPP, and 15% starch. The barrel screw speed was set at a constant speed of 300 rpm and the diameter of the die opening was 4.08 mm. The flour was fed into the extrusion barrel at 1.3 kg/min. The barrel temperature was established into four different temperature zones; 60/80/90/110 °C. The extrudates were dried in an oven at 103 °C for 10 min.

2.3. bulk density

A graduated cylinder was filled with dry TPP by gentle tapping twice to eliminate the interspace between the crumbles. The volume and weight were recorded, and the bulk density was calculated as the weight per volume (g/L).

2.4. Water- and oil-absorption capacities

The liquid absorption capacities (water and oil absorption capacities) were evaluated (Sakai et al., 2023) by dissolving TPP in deionized water or canola oil (1:20 solid-to-liquid ratio), followed by vortexing for 30 s. After incubation for 30 min at 25 °C, the mixture was centrifuged at 2000×*g* at 25 °C for 10 min. The precipitate and supernatant were weighed, and the liquid-holding capacities were calculated in grams of water or oil retained per gram of protein.

2.5. Color analysis

The color of TPP was determined using a colorimeter (Chroma Meter CM-700d/600d; Konica Minolta, Tokyo, Japan) (Sakai et al., 2022). The results of the color analysis were expressed according to the Commission International de l'Eclairage system and reported as Hunter L* (lightness), *a** (redness), and *b** (yellowness) values.

2.6. Amino acid and peptide analysis

The amino acids and peptides were extracted from crushed TPPs via agitation with artificial saliva (Brodkorb et al., 2019) at 37 °C for 5 min. The obtained amino acids were quantified using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, United States) equipped with a Zorbax Eclipse-AAA column (4.6 × 150 mm, Agilent). The mobile phase comprised a 20 mM Na_2HPO_4 buffer (pH 8.2) and a 45:45:10 methanol:acetonitrile:H_2O mixture at a flow rate of 0.65 mL/min for 40 min (Sakai et al., 2024c). The peptides and furan-bound peptides were detected at 220 and 420 nm, respectively, using an HPLC (Nexera X2, Shimadzu, Kyoto, Japan) equipped with a UV detector. The peptides were separated using a TSKgel G2000SWXL column (300 × 7.8 mm I.D., Tosoh Corporation, Tokyo, Japan) with isocratic elution of 45% acetonitrile containing 0.1% trifluoroacetic acid for 25 min at a flow rate of 0.7 mL/min at 40 °C. The peptides were desalted for sensory evaluations using Pierce C18 Tips (Thermo Fisher Scientific Inc., Massachusetts, USA). To determine the amino acid sequence of peptides, the purified peptides were immobilized on a glass fiber disk and identified via Edman

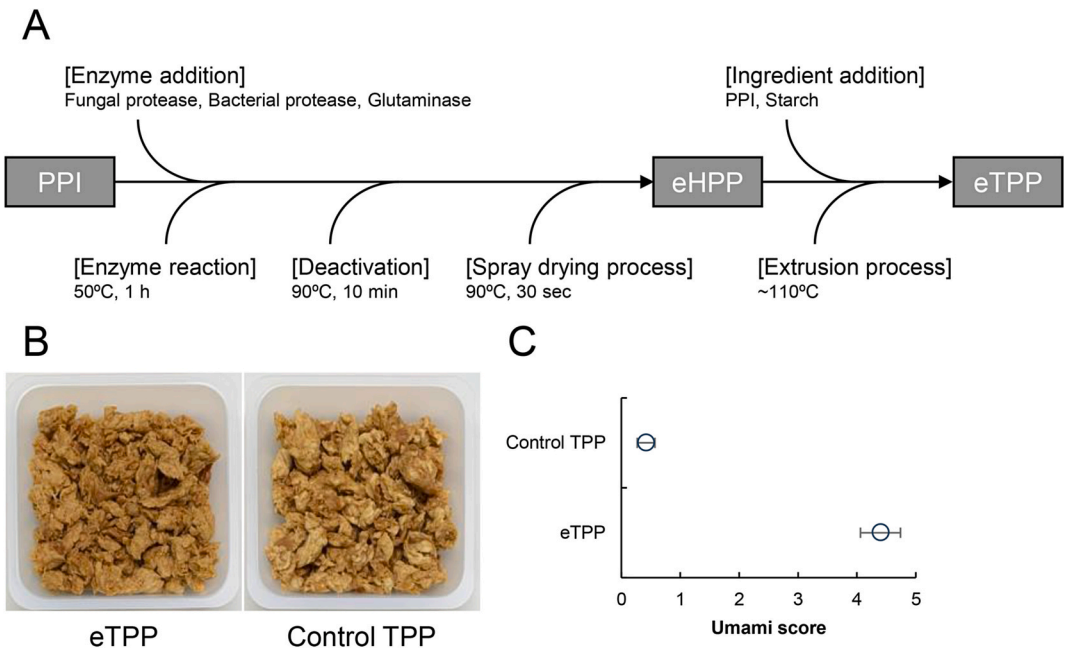

Fig. 1. Appearance of the control TPP and eTPP. (A) Manufacturing process of eTPP. (B) Appearance of eTPP and the control TPP. (C) Umami scores of TPPs evaluated via simple sensory tests.

degradation using a protein sequencer PPSQ-31A (Shimadzu, Kyoto, Japan), according to the manufacturer's instructions. The peptide and isopeptide bonds were degraded via acid hydrolysis using 6 M HCl and autoclaving at 120 °C for 2 h (Araya et al., 2021). After the addition of diethyl ether to the hydrolysate solutions, the amino acids and HMF were extracted in the aqueous and diethyl ether layers, respectively.

2.7. Preparation of PBMA patties

To evaluate the effect of eTPP on the sensory properties of PBMA patties, the patties were prepared using TPP (20 g), water (20 g), methylcellulose (2 g), canola oil (2 g), potato starch (2 g), and PPI (1 g). In the production process involving washing, the dried TPP was immersed in water (1:1 mass-to-volume ratio) for 2 h for hydration, followed by dehydration. The washing process was repeated three times and the swollen TPPs were dehydrated to a constant weight of 40 g. In the production process without washing, the dried TPP was immersed in water (1:1 mass-to-volume ratio) for 2 h for hydration, and the swollen TPP weighed 40 g. The hydrated TPP was mixed with methylcellulose, potato starch, PPI, and canola oil. The samples were blended for 60 s using a hand blender and the patty dough was molded (60 × 40 × 25 mm). The dough was cooked at 180 °C for 15 min and then cooled to 50 °C before further analysis.

2.8. Protein surface hydrophobicity measurement

Surface hydrophobicity was measured using 8-anilino-1-naphthalenesulfonic acid (ANS) as the fluorescent probe. The protein powders were mixed with 50 mM phosphate buffer (pH 7.0) to obtain protein solutions at concentrations ranging between 0.01 and 1.0 mg/mL. Then, 20 μL of 8 mM ANS was added to 2 mL of the sample solutions. The hydrophobicity index was obtained from the initial slope of the fluorescence intensity versus the protein concentration plot.

2.9. Analysis of beany off-flavor volatilization

The volatilization amounts of the beany off-flavor compounds were quantified using headspace solid-phase microextraction-gas chromatography/mass spectrometry equipped with polydimethylsiloxane as the solid-phase microextraction fiber (HS-SPME-GC/MS, Shimadzu, Kyoto, Japan), as described in a previous study (Sakai et al., 2023). 1,2-Dichlorobenzene (1.0 mg/g-patty) was used as the internal standard.

2.10. Texture profile analysis

Texture profile analysis (TPA) of the parameters of PBMA patties (cut into 10 mm × 10 mm × 10 mm pieces) was conducted using a rheometer (COMPAC-100II; Sun Scientific Co., Ltd., Tokyo, Japan) equipped with a flat cylindrical probe with a diameter of 20 mm as described in a previous study (Sakai et al., 2024b).

2.11. Sensory evaluation

The sensory taste of the meat analog patties was evaluated by 12 employees (seven men and five women aged 25–55 years) of Amano Enzyme Inc. trained in sensory evaluation. These sensory panelists were selected based on their ability to perceive the five basic tastes (umami, saltiness, sweetness, bitterness, and sourness) according to ISO 8586:2023 (ISO 8586, 2023). The term kokumi was defined as perceived thickness, mouthfulness, continuity, and depth, in accordance with a previous report (Yamamoto and Inui-Yamamoto, 2023). These terms are defined in ISO 8586:2023 and ISO 5492:2008 (ISO 8586, 2023; ISO 5492, 2008). The simple sensory taste was scored on an unstructured scale from 0 (tasteless or flavorless) to +5 (strong taste or flavor) for the umami levels of TPP and the effects of the washing process on the sensory profiles of TPP-based patties. The detailed sensory taste of the TPP-based patties was scored on a structured scale from −2 (weak) to +2 (strong) relative to that of the control patty, which was scored as 0. The saltiness ratio of the eTPP-based patties was scored on an unstructured scale relative to that of the control patty, for which the ratio was 1. The sensory panelists were trained using a NaCl solution in 0.05% increments.

2.12. Electronic tongue analysis

The umami and saltiness tastes of the PBMA patties were quantified using a TS-5000Z electronic tongue (Intelligent Sensor Technology Inc., Tokyo, Japan). Such devices are employed for the analysis of various

final food products or ingredients, to reveal the umami or saltness intensity ratio and their synergistic enhancing effects (Chen et al., 2023; Sakai et al., 2025; Wang et al., 2024). The sensors attached to the measurement device contain an artificial lipid membrane and positive and negative reference electrodes and collect taste characteristics through AAE sensor (umami) or CTO sensor (saltiness). All samples were measured at room temperature (25 ± 2 °C). The measurement samples were mixtures of 5 g water and 5 g PBMA patties or crushed TPP, and were scored on a structured scale from −2 (weak) to +2 (strong) relative to the control patties or crushed control TPP, which was assigned a score of 0.

2.13. Sodium ion analysis

The sodium ions were quantified using a LAQUAtwin-Na-11 pocket meter (Horiba Ltd., Kyoto, Japan). Standard curves of sodium were generated at 25 °C using 0.01, 0.1, 1, 2.0, and 2.5% NaCl solutions. The plant-based patties were crushed, mixed, and soaked in artificial saliva at 37 °C for 5 min. Subsequently, the mixtures were centrifuged at 15,000×*g* for 10 min, and the salt concentrations in the supernatants were analyzed.

2.14. Glucose and 5-hydroxymethylfurfural analysis

Glucose (Glc) and 5-hydroxymethylfurfural (HMF) were extracted from crushed TPPs via agitation with deionized water or diethyl ether at 37 °C for 30 min. After filtering through a membrane filter (pore size 0.45 μm), the solution containing Glc and HMF was injected into the HPLC instrument, equipped with an MCI GEL CK04S (300 × 8.0 mm I.D.; Mitsubishi Chemical Corporation, Tokyo, Japan) connected to a refractive index detector. The mobile phase comprised MilliQ water at a flow rate of 0.4 mL/min and the analysis was conducted at 80 °C for 30 min. Standard curves were prepared using solutions containing different concentrations of Glc and HMF.

2.15. In vitro digestion

In vitro digestion was performed using the INFOGEST digestion method (Brodkorb et al., 2019). The finely crushed PBMA patties were treated with oral, gastric, and intestinal digestive enzymes. Samples from all three phases were boiled at 100 °C for 5 min. The free amino nitrogen content was measured using the ninhydrin method (Sakai et al., 2023).

2.16. Ethical permission

All experiments were performed in accordance with the relevant guidelines and regulations of Amano Enzyme, Inc. The Research Commission of Innovation Divisions at Amano Enzyme, Inc. approved the procedures and methodologies related to the sensory evaluation of this study (Approval No: 20230224). Consent was obtained from all participants before the sensory evaluation.

2.17. Statistical analysis

The obtained data are presented as the mean ± standard error of three independent experiments to evaluate the effects of the different sample formulations and preparations. The statistical differences among the multiple groups were determined using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The statistical differences between the curves were analyzed using two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Tukey's test at significance levels of 95% ($p < 0.05$) and 99% ($p < 0.01$) was used to determine the significant differences between the results. Heatmap and clustering analyses were performed using Heatplus in Bioconductor software (version 3.19).

3. Results and discussion

3.1. Production and characterization of eTPP

We first investigated the adverse effects of eHPP on the color and physical properties of TPP. Fig. 1B shows the appearance of the control TPP and eTPP, with eTPP being slightly brown compared with the control TPP. To investigate the color properties, the objective color attributes of PPI, eHPP, and the two TPPs were characterized using the $L^*a^*b^*$ coordinates (Table 1). The a^* values of all pea proteins were similar, however, the L^* and b^* values of PPI and eHPP, control TPP, and eTPP decreased in stages, indicating that eTPP is browner. Generally, this browning reaction of PPI or TPP is induced by chemical reactions such as the Maillard reaction, caramelization, or polymerization, involving interactions among reducing sugars, proteins or amino acids, and phenolic compounds (Wu et al., 2024). Furthermore, the browning degrees depend on the reactant amounts (Wu et al., 2024). Such findings suggest that the brown intensities of eTPP are enhanced because of the increase of amino acids and peptides via enzymatic hydrolysis and the presence of 13.1% Glc in starch. The L*a^*b^* coordinates revealed that eTPP had a slight brown color; however, its strength was not considered unacceptable by consumers compared with previous reports (Lee et al., 2022; Peñaranda et al., 2023; Sakai et al., 2022; Wu et al., 2024). The general physical properties of the two TPPs were then determined next (Table 1). The bulk density and water-/oil-absorption capacity of eTPP were similar to those of the control TPP. Moreover, as shown in Fig. 1C, sensory evaluation indicated that the umami score of eTPP was significantly higher than that of the control TPP. Therefore, eTPP had better sensory properties without compromising its color and physical characteristics.

To investigate the pea protein hydrolysates produced by proteases and glutaminase, the amino acids and peptides released from TPP in artificial saliva were analyzed via HPLC (Fig. 2). The amount of free amino acids from eTPP was significantly increased compared with that from the control TPP, excluding Gln (Fig. 2A). In particular, the Glu content of the control TPP was 0.05 mmol/g, which was below the Glu threshold for humans for perceiving umami (0.06 mmol/g) (Delompré et al., 2019). Conversely, the Glu content of eTPP was 6.57 mmol/g, which was above the umami threshold. Fig. 2B shows the heatmap and cluster analysis of the increased rate of eTPP compared with the amino acid content in the control TPP. The rates of Glu, Arg, Lys, Ser, Phe, Val, and Ala released from eTPP were notably higher than those released from the control TPP. Size-exclusion chromatography was performed to separate the peptides released from TPPs, revealing four peaks (P1, P2, P3, and P4) for eTPP, with estimated molecular weights of 0.28, 0.35, 0.57, and 0.88 kDa, respectively (Fig. 2C). Remarkably, only purified P3 displayed a slight brown color, whereas the other purified peptides were white. These findings indicate that proteases produce substantial amounts of specific amino acids and lower molecular weight peptides from pea proteins and glutaminase deamidated free Gln to Glu, with an umami taste.

The types and amounts of free amino acids and peptides affect food

Table 1
Physicochemical and color properties of TPP.

	PPI	eHPP	Control TPP	eTPP
L^* value	69.8 ± 0.6[a]	69.6 ± 0.5[a]	66.8 ± 0.7[b]	63.2 ± 0.7[c]
a^* value	6.4 ± 0.3[a]	6.3 ± 0.1[a]	6.3 ± 0.2[a]	6.4 ± 0.1[a]
b^* value	24.2 ± 0.2[a]	24.3 ± 0.3[a]	22.1 ± 0.4[b]	19.6 ± 0.4[c]
Bulk density (g/mL)	–	–	2.9 ± 0.2[a]	2.8 ± 0.1[a]
Water absorption capacity (g/g-TPP)	–	–	1.49 ± 0.09[a]	1.35 ± 0.07[a]
Oil absorption capacity (g/g-TPP)	–	–	0.36 ± 0.02[a]	0.39 ± 0.04[a]

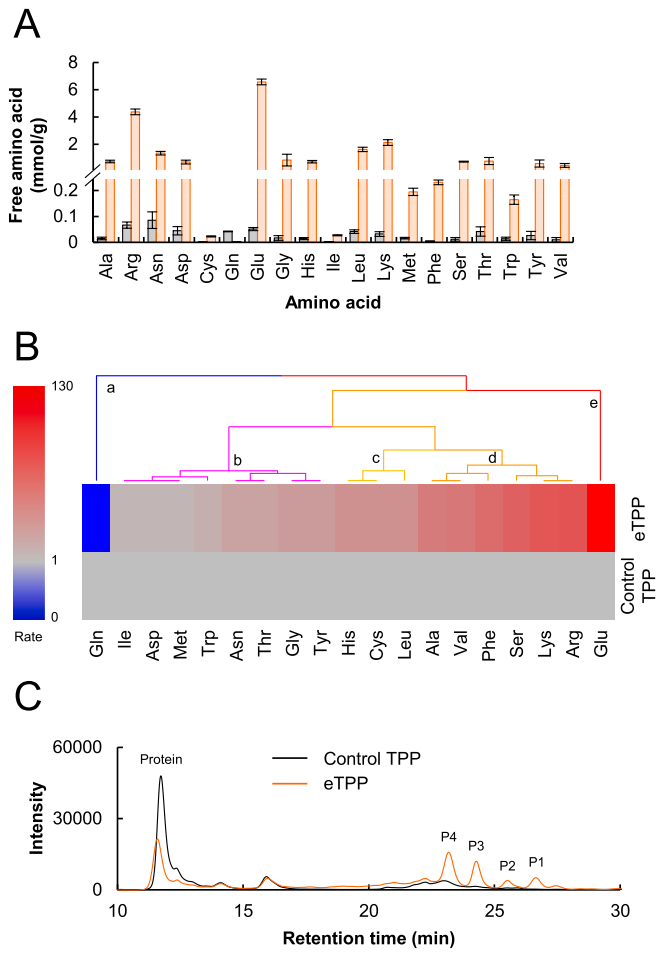

Fig. 2. Compositional analysis of protein hydrolysates in TPP. HPLC analysis (A) and heatmap and cluster analysis (B) of the free amino acids released from TPP in artificial saliva. The relative free amino acid compositions were clustered into five different groups according to Euclidean distance: (a) significantly lower cluster, (b) slightly higher cluster, (c) higher cluster, (d) substantially higher cluster, and (e) substantially highest cluster. (C) Size exclusion chromatography of the free peptides released from TPP in artificial saliva. The error bars represent the mean ± standard error of three independent experiments.

palatability (Delompré et al., 2019; Diepeveen et al., 2022; Dai et al., 2022). Of the 18 amino acids increased by the enzyme blend, 12 had a bitter taste, 4 had a sweet taste, and 2 had an umami taste (Delompré et al., 2019). However, the concentrations of all 18 amino acids except Glu, Arg, and Lys were lower than the thresholds for sensing bitter, sweet, and umami tastes (Delompré et al., 2019), respectively. Therefore, it is suggested that among the enzyme-produced amino acids, the umami taste of eTPP was attributed to the Glu molecules. Four peptides (P1, P2, P3, and P4) released from eTPP were separated and collected, followed by sensory evaluation of each peptide (Table 2). The enzyme-produced peptides did not enhance the umami taste of eTPP, however, P1, P2, P3, and P4 exhibited astringent, bitter, slightly bitter, and slightly sour tastes, respectively. Generally, because of the relatively high proportion of hydrophobic amino acids in pulse-derived proteins, enzymatically hydrolyzed peptides can elicit a bitter or astringent taste (Sun et al., 2022a; Lei et al., 2019). This suggests that the proteases used in this study produced hydrophobic peptides with a slightly unpleasant taste.

Table 2
Purified peptide contents and their taste characteristics.

Peptide	Content (mg/g-eHPP)	Taste characteristics
P1	91.4	Astringent, sour
P2	43.7	Bitter, slightly spicy
P3	147.2	Slightly bitter
P4	167.9	Slightly sour, astringent

3.2. *Effects of the wash process on the sensory profiles of PBMA patties*

The effects of the wash process on the physical and sensory properties of PBMA patties were investigated (Fig. 3A). Texture profile analysis of the control and eTPP-based patties revealed no significant differences between the inclusion or exclusion of the washing process (Table 3). To investigate the effects of the washing process on the sensory profiles of eTPP-based patties, the Glu content and n-hexanal volatilization amount were measured. In comprehensive reviews, Glu and n-hexanal molecules are considered indicators of the umami taste and beany off-flavor (Trindler et al., 2022; Zhao et al., 2019). The Glu contents of the eTPP-based patties were above the threshold for perceiving umami by humans (0.06 mmol/g) (Delompré et al., 2019), whereas those of the control patties were below the umami threshold (Fig. 3B). Interestingly, the n-hexanal amounts released from the eTPP-based patties were significantly lower than those released from the control patties (Fig. 3C). In addition, the washing process reduced the contents of Glu and n-hexanal in both patties (Fig. 3B and C). Subsequently, the sensory evaluations of the PBMA patties without the washing process also showed that the eTPP-based patties had significantly higher umami scores and lower beany flavor scores than the control patties (Fig. 3D). Furthermore, similar to the results of instrumental analysis (Fig. 3B and C), the washing process reduced the umami and beany flavor scores of both patties (Fig. 3E). Remarkably, the beany flavors of the eTPP-based patties without the washing process were equal to or lower than those of the control patties with the washing process, suggesting that the washing process could be omitted in eTPP, while relieving the beany off-flavors of PBMA patties. This could overcome the technical challenges associated with the wash process of pea-based extrudates and further reduce the costs and production time.

Another challenge is the off-flavor of pulse beans, which affects the overall flavor of PBMA products and restricts consumer acceptability (Lao et al., 2024). Pea off-flavor is related to 20 volatile compounds that are above the odor threshold (Lao et al., 2024). They are divided into fatty aldehydes, fatty alcohols, fatty ketones, furan derivatives, and aromatic compounds (Trindler et al., 2022). In particular, the components contributing to the pea off-flavor are mainly n-hexanal, 2-octenal, nonanal, 1-octen-3-ol, and 2-pentylfuran (Trindler et al., 2022). These flavor substances bind to protein molecules via non-covalent interactions, particularly hydrophobic interactions (Wang and Arntfield, 2015, 2017). It is assumed that the hydrolysis of pulse-derived proteins leads to the loss of integrity of the protein hydrophobic region by disorganizing the tertiary structure of proteins, thereby loosening the interactions between the proteins and flavor substances (Wang and Arntfield, 2015; Tang et al., 2009). Thus, we investigated the effects of the eTPP production process on the degree of hydrophobicity and hexanal amount of pea proteins (Fig. 3F). Consequently, the relative hydrophobicity of proteins was decreased via enzyme-catalyzed degradation, while the amount of hexanal in proteins decreased upon heat treatment after the enzyme-catalyzed degradation.

Also, the amounts of other beany off-flavor compounds were quantified by HS-SPME-GC/MS. As a result, the amounts of nonanal, 2-octenal, 1-octen-3-ol, 2-pentylfuran, benzaldehyde, and pyrazine were 2.07, 1.38, 3.05, 0.54, 0.46, and 0.07 μg/g in the control PPI. Similar to hexanal, the amounts of these compounds also decreased after heat treatment of eHPP (Fig. 3G). This indicated that the amount of other off-flavor compounds as well as n-hexanal decreased upon heat treatment after the enzyme-catalyzed degradation. Namely, the reduction of the beany off-flavor levels of eTPP (Fig. 3D and E) could be achieved by

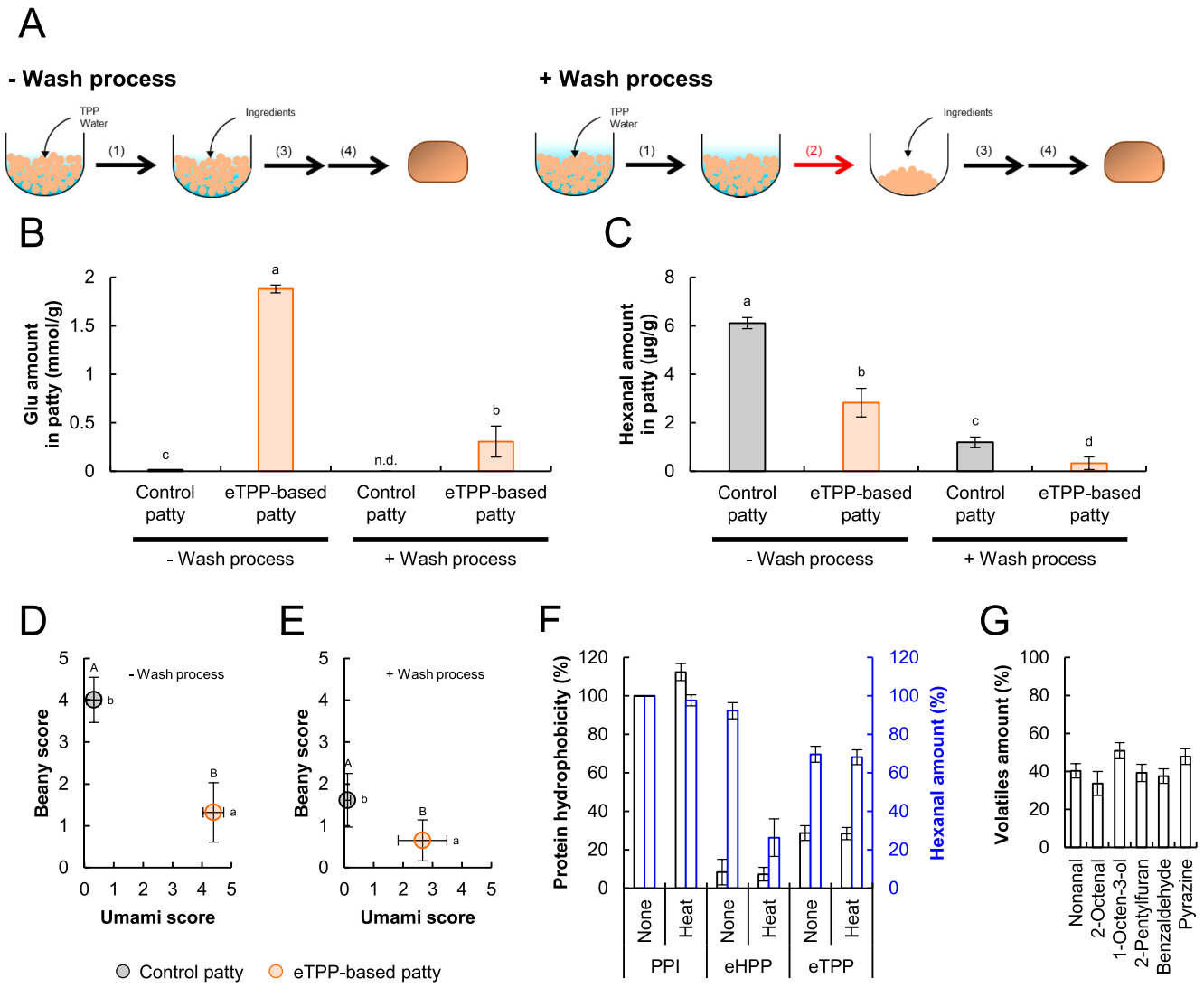

Fig. 3. Effects of the wash process on the sensory profiles of PBMA patties. (A) Manufacturing flow charts of the PBMA patties with and without the wash process. (1) Hydration and soaking; (2) dehydration; (3) ingredient addition; (4) grill. (B, C) Effects of the wash process on Glu amounts (B) and hexanal volatilization (C) from PBMA patties. (D, E) Effects of the wash process on the umami (D) and beany (E) scores of sensory evaluations. (F) Protein hydrophobicity and hexanal amount of PPI, eHPP, and eTPP. (G) Amounts of the beany off-flavor components released from eHPP. The error bars represent the mean ± standard error of three independent experiments.

Table 3
Texture profile analysis of TPP-based PBMA patties.

	Wash process	Hardness (N)	Cohesiveness	Springiness	Chewiness (N)
Control patty	+	11.4 ± 0.73^{a}	0.86 ± 0.05^{a}	0.84 ± 0.03^{a}	8.24 ± 0.46^{a}
	–	11.5 ± 0.45^{a}	0.89 ± 0.03^{a}	0.85 ± 0.05^{a}	8.70 ± 0.68^{a}
eTPP-based patty	+	11.7 ± 0.58^{a}	0.88 ± 0.08^{a}	0.83 ± 0.11^{a}	8.45 ± 0.48^{a}
	–	11.8 ± 0.64^{a}	0.90 ± 0.05^{a}	0.84 ± 0.08^{a}	8.82 ± 0.84^{a}

reducing various off-flavor compounds induced by heat treatment after the enzyme-catalyzed degradation. In general, heat treatment of protein hydrolysates hydrates the hydrophobic peptides and increases their solubility, thus decreasing the binding ability of flavor compounds to the protein hydrolysates (Saffarionpour, 2024; Wang and Arntfield, 2015, 2017). Therefore, the release of flavor compounds (e.g., n-hexanal, nonanal, 1-octen-3-ol, and benzaldehyde) from protein hydrolysates (e.g., soy, pea, pork, and fish proteins) is promoted (Flores et al., 2024; Li et al., 2022; Shi et al., 2022; Wang et al., 2022). In addition, heat treatment increases the kinetic energy of flavor compounds and overcomes the intermolecular attractive forces with other components, rapidly increasing the volatilization rate (Chen et al., 2023; Zhang et al., 2021). Thus, it was suggested that heat treatment after protein hydrolysis could promote the volatilization amount and rate of beany off-flavor compounds during the eTPP production process by decreasing the interaction of off-flavor compounds and protein hydrolysates. Such treatment could afford eTPP-based patties with lower beany off-flavor levels.

3.3. Sensory evaluation of eTPP-based patty with and without salt

The sensory properties of the eTPP-based PBMA patties that skipped

the washing step were assessed (Fig. 4). Fig. 4A shows that the umami and kokumi levels of the eTPP-based patty were significantly higher ($p < 0.01$) than those of the control patty. Although Arg, Lys, P2, and P3 in eTPP exhibit bitter tastes, the bitterness scores of both patties were approximately the same. This is because the umami enhancement by the Glu molecules can mask the intensity of bitterness (Wang et al., 2020). Next, we evaluated the effects of salt on the sensory profiles of the eTPP-based PBMA patties (Fig. 4B). The umami and kokumi levels of the eTPP-based patties with 0.5% NaCl were significantly higher than those of the control patties with 0.5% NaCl ($p < 0.01$). Conversely, the bitterness levels were not significantly different between the patties ($p > 0.05$). This result was consistent with that of the TPP-based patties without NaCl addition (Fig. 4A). Interestingly, 0.5% NaCl supplementation significantly enhanced the saltiness of the eTPP-based patties ($p < 0.01$) compared to that of the control patties (Fig. 4B). Furthermore, the electronic tongue analysis showed similar results to those of the sensory evaluations (Fig. 4C). Therefore, the protein hydrolysates (amino acids or peptides) in eTPP could enhance the saltiness level of NaCl in the PBMA patties in addition to the umami and kokumi levels.

The salt reduction rate of the eTPP-based patties was investigated and compared with that of the control patties with NaCl supplementation (Fig. 4D). In the sensory evaluation, the saltiness intensity ratio of the control patties with 0.50% NaCl was set to 1.0, while the intensity ratios of the eTPP-based patties with 0.50%, 0.45%, 0.40%, and 0.35% NaCl were 1.27, 1.13, 0.96, and 0.85, respectively. The saltiness intensity of the eTPP-based patties supplemented with 0.50% and 0.45% NaCl was significantly enhanced ($p < 0.01$) compared to that of the control patties with 0.50% NaCl. The eTPP-based patties supplemented with 0.40% NaCl were comparable to the control patties supplemented with 0.50% NaCl. These results were consistent with the electronic tongue analysis results (Fig. 4E). As expected, a positive correlation was observed between the amount of NaCl added and the residual amount of NaCl after the cooking process (Fig. 4F). These findings suggest that the eTPP-based patties consisting of pea proteins treated with proteases and glutaminase could be an effective strategy for achieving a salt reduction rate of 20%.

The salt reduction rates of saltiness-enhancing substances are crucial indicators for developing practical sodium-reduced food products (Le et al., 2022). Previous studies reported salt reduction rates equal to or higher than the 20% achieved in this study (Fig. 4D), including a 60%

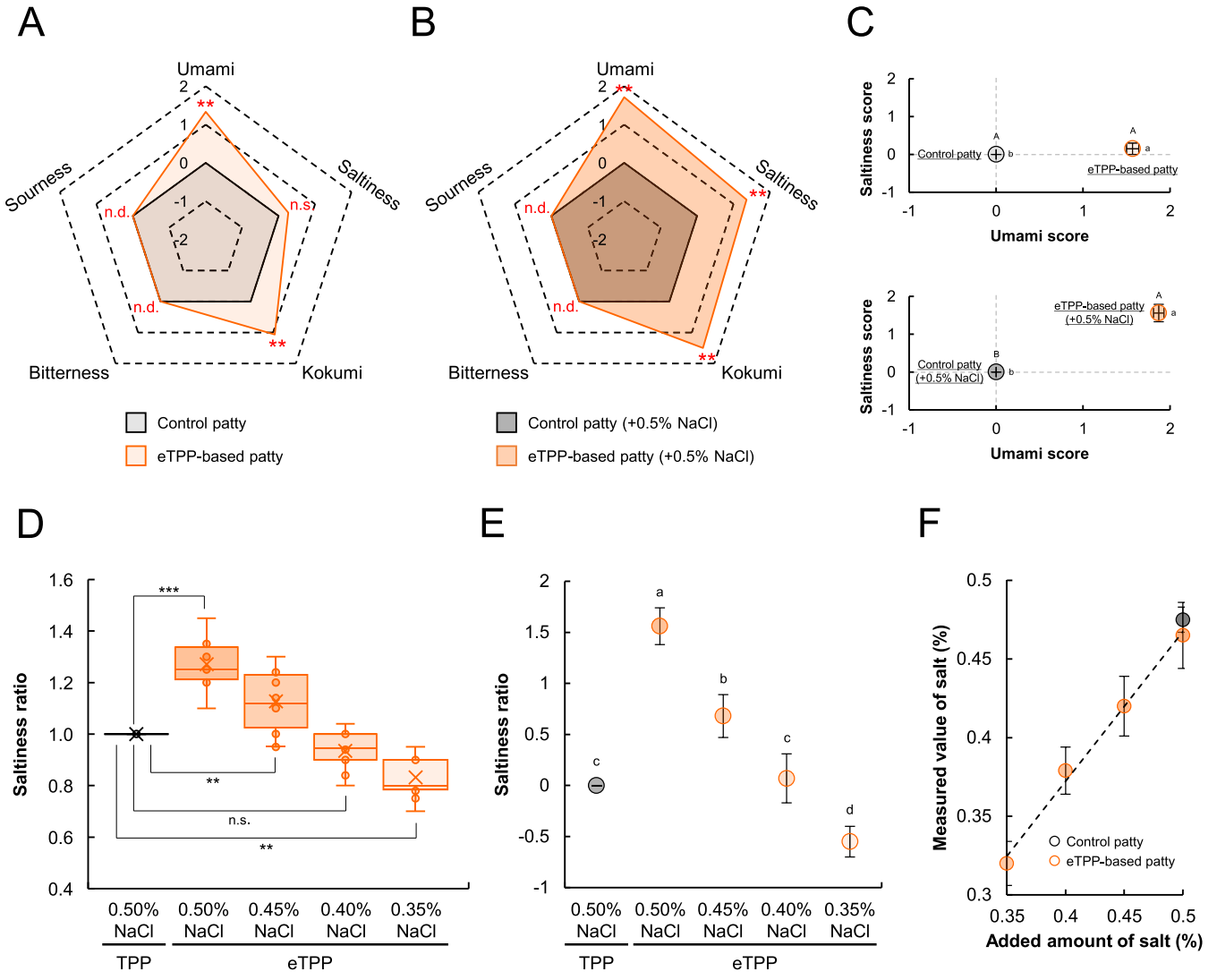

Fig. 4. Sensory profiles of the PBMA patties with and without salt. (A, B) Sensory evaluations of the control and eTPP-based patties in the absence (A) and presence (B) of 0.5% NaCl. n.s., not significant; n.d., not detected. (C) Electronic tongue analysis of the control and eTPP-based patties in the absence and presence of 0.5% NaCl. (D, E) Salt reduction rate of the control and eTPP-based patties. Sensory evaluations (D) and electronic tongue analysis (E) of eTPP-based patties in the presence of 0.35%, 0.40%, 0.45%, and 0.50% NaCl, compared with the saltiness of PBMA patties in the presence of 0.50% NaCl. (F) NaCl contents in the PBMA patties. The error bars represent the mean ± standard error of three independent experiments. Different letters indicate significant differences ($p < 0.05$).

reduction by the Glu additive (Hayabuchi et al., 2020), 30% reduction by octapeptides derived from mushrooms (Wang et al., 2024), 25% reduction by hydrolysates derived from mushrooms (Chen et al., 2023), and 20% reduction by umami nucleotides contained in yeast extracts (Desmond, 2006). Few are the studies on the salt reduction rates in PBMA products, with one reporting that the addition of disodium succinate resulted in a 24% salt reduction (Sun et al., 2022b). Even though the addition of such substances to final food products can be an attractive strategy for salt reduction, their feasibility or versatility might be limited due to production or purification costs, taste due to contaminants, and legal regulations as food additives. Such strategies fail to meet the clean-label requirements, increasing consumers' tendency to avoid products containing food additives (Asioli et al., 2017). Food-grade enzymes and their reaction products are not considered additives, due to enzymes being deactivated during processing or grilling. In this study, proteases and glutaminase did not exhibit any enzymatic activities after the eHPP production process (data not shown). Therefore, eTPP produced by proteases and glutaminases could be a novel strategy to improve the saltiness of PBMA products, while meeting clean-label requirements.

3.4. Exploration of eTPP-contained hydrolysates enhancing saltiness

The enzyme-produced amino acids in eTTP were screened for their saltiness enhancement effects (Fig. 5). In a preliminary study, all amino acids were added to the control TPP with NaCl and substantially enhanced its saltiness levels, suggesting that amino acids that can enhance saltiness exist in eTPP with NaCl (data not shown). Subsequently, eight amino acids (Glu, Arg, Lys, Ser, Phe, Ala, Val, and Leu) were selected based on the results in the amounts (Fig. 2A) and relative increase ratios (Fig. 2B) of amino acids in eTPP. The addition of eight amino acids and NaCl similarly enhanced the saltiness levels of the control TPP. Conversely, the other 11 amino acids did not show any saltiness-enhancing effect (data not shown). The interaction between amino acids that exhibit certain tastes with taste receptors commonly depends on their properties (e.g., structure, polarity, and charge) (Delompré et al., 2019). Thus, the eight amino acids were classified according to their chemical and taste properties, and the saltiness levels of the control TPP containing amino acids and NaCl in various patterns were evaluated (Fig. 5A). Supplementation with Glu, Arg, and Lys significantly enhanced the saltiness level of the control TPP with NaCl and was comparable to the supplementation with all eight amino acids. These findings suggest that the presence of Glu, Arg, and Lys is a crucial factor in enhancing the saltiness of eTPP with NaCl. However, the saltiness intensity ratio of the control TPP supplemented with Glu, Arg, and Lys was significantly lower than that of eTPP, suggesting that factors other than amino acids may exist in eTPP.

Subsequently, we screened the enzyme-produced peptides in eTPP that exhibited saltiness enhancement effects. The enzyme-produced peptides were mixed with 0.5% NaCl, and their saltiness levels were compared with 0.5% NaCl (Table 4), revealing that only P3 enhanced the saltiness intensity. Sensory evaluations were then conducted for the control TPP, including Glu, Arg, Lys, and NaCl, with different peptide patterns (Fig. 5B). Supplementation with P1, P2, and P4 did not change the saltiness level of the control TPP, including Glu, Arg, Lys, and NaCl, whereas supplementation with P3 significantly increased the saltiness levels. Notably, the saltiness intensity levels of the control TPP, including Glu, Arg, Lys, P3, and NaCl, were comparable to those of eTPP, including NaCl. Furthermore, the electronic tongue analysis revealed similar results (Fig. 5C). These findings suggest that the combination of Glu, Arg, Lys, and P3 may be an important factor in enhancing the saltiness intensity of NaCl.

Saltiness perception is expressed through interactions of certain substances with several receptors and ion channels, especially epithelial sodium channels (ENaCs), transient receptor potential vanillic acid 1 (TRPV1), and the transmembrane channel-like 4 (TMC4) (Le et al., 2022; Wang et al., 2024; Chen et al., 2023). However, the saltiness enhancement mechanisms at the molecular level are understudied (Le et al., 2022). Recently, molecular docking simulations and sensory evaluations revealed that negatively charged peptides containing Glu can interact with the TMC4 receptors, similar to chloride ions, thereby enhancing the saltiness intensity of NaCl (Shen et al., 2022; Xie et al., 2023). Moreover, using cultured human fungiform taste papillae cells, Xu et al. (2017) reported that basic amino acids such as Lys and Arg bind to the ENaC receptor, thereby increasing the frequency and intensity of NaCl-induced responses and enhancing the saltiness perception of NaCl (Xu et al., 2017). Therefore, we suggest that the enzyme-produced Glu

Table 4
Saltiness intensity of eTPP-contained peptides with NaCl.

Peptides	Saltiness levels compared with NaCl
P1 + NaCl	No change
P2 + NaCl	No change
P3 + NaCl	Strong saltiness
P4 + NaCl	No change

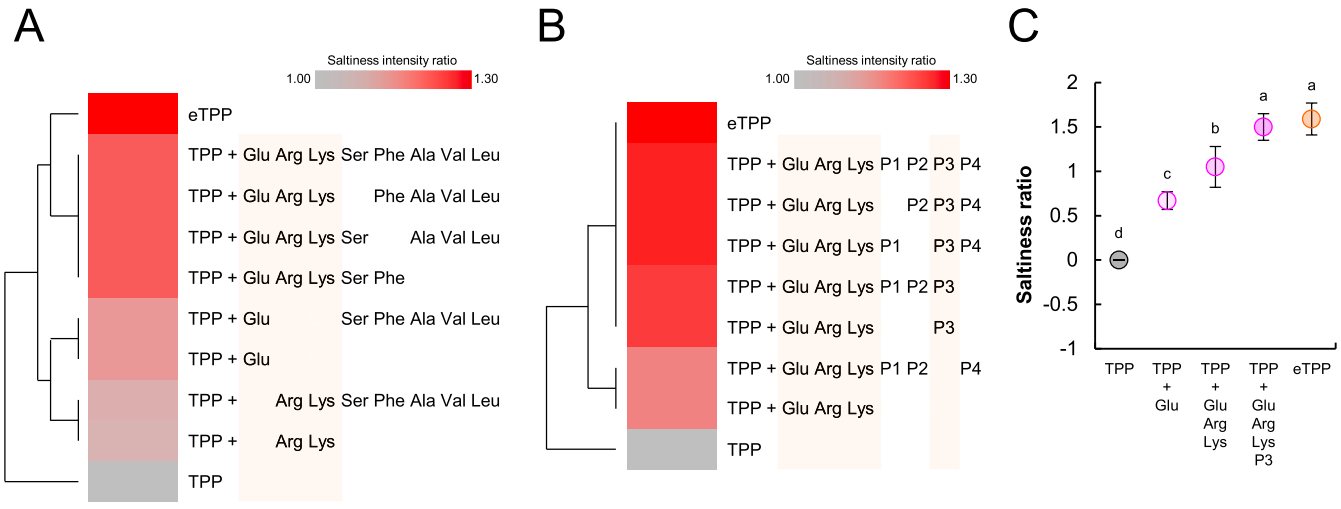

Fig. 5. Screening saltiness-enhancing hydrolysates. (A, B) Exploration of saltiness-enhancing amino acids (A) and peptides (B). Amino acids and peptides were added to the control TPP at the same concentrations as in eTPP. (C) Saltiness-enhancing effects of Glu, Arg, Lys, and P3 on control TPP via electronic tongue analysis. The error bars represent the mean ± standard error of three independent experiments. Different letters indicate significant differences ($p < 0.05$).

and Arg + Lys could interact with the TMC4 and ENaC receptors, thereby enhancing the saltiness perception of NaCl.

3.5. Characterization of the saltiness-enhancing P3 components

The amino acid sequence of the saltiness-enhancing peptide, brownish P3, could not be identified via Edman degradation (data not shown). Thus, considering that P3 was chemically modified until eTPP production, the free peptides from each process were analyzed via gel-filtration chromatography (Fig. 6A). None of the peaks changed during the eHPP production process (after the enzyme reaction, deactivation treatment, and spray drying of eHPP). During the eTPP production process (after extrusion of the mixture of eHPP and starch), the P2 peak significantly decreased and the P3 peak accordingly increased compared with the eHPP production process. Thus, the extrusion process converted P2 into P3. Identification of the amino acid sequence of P2 via Edman degradation revealed a sequence of Glu-Gly-Lys-Gly (molecular weight 0.39 kDa) coded in one of the main storage proteins, convicilin, derived from pea (*Pisum sativum*). Moreover, after eTPP production, only P3 enhanced the saltiness of NaCl (Fig. 6B) and strengthened its brown color intensity (data not shown). These findings suggest that a Maillard reaction may have caused P3 production because the extrusion process of eHPP in this study was conducted at an extremely high temperature in the presence of starch which contained 13.1% Glc.

Maillard-reacted peptides are generally produced via the condensation of the carbonyl group of HMF, formed by the dehydration of monosaccharides at an early stage of the Maillard reaction, with the ε-amino group of Lys residues, constituting brown peptides with furan-bound Lys residues (Kutzli et al., 2021). Measurements of the Glc and HMF contents before and after the extrusion process (Fig. 6C and D) revealed a decrease in the Glc content, whereas the HMF content accordingly increased in the control TPP after the extrusion process. In

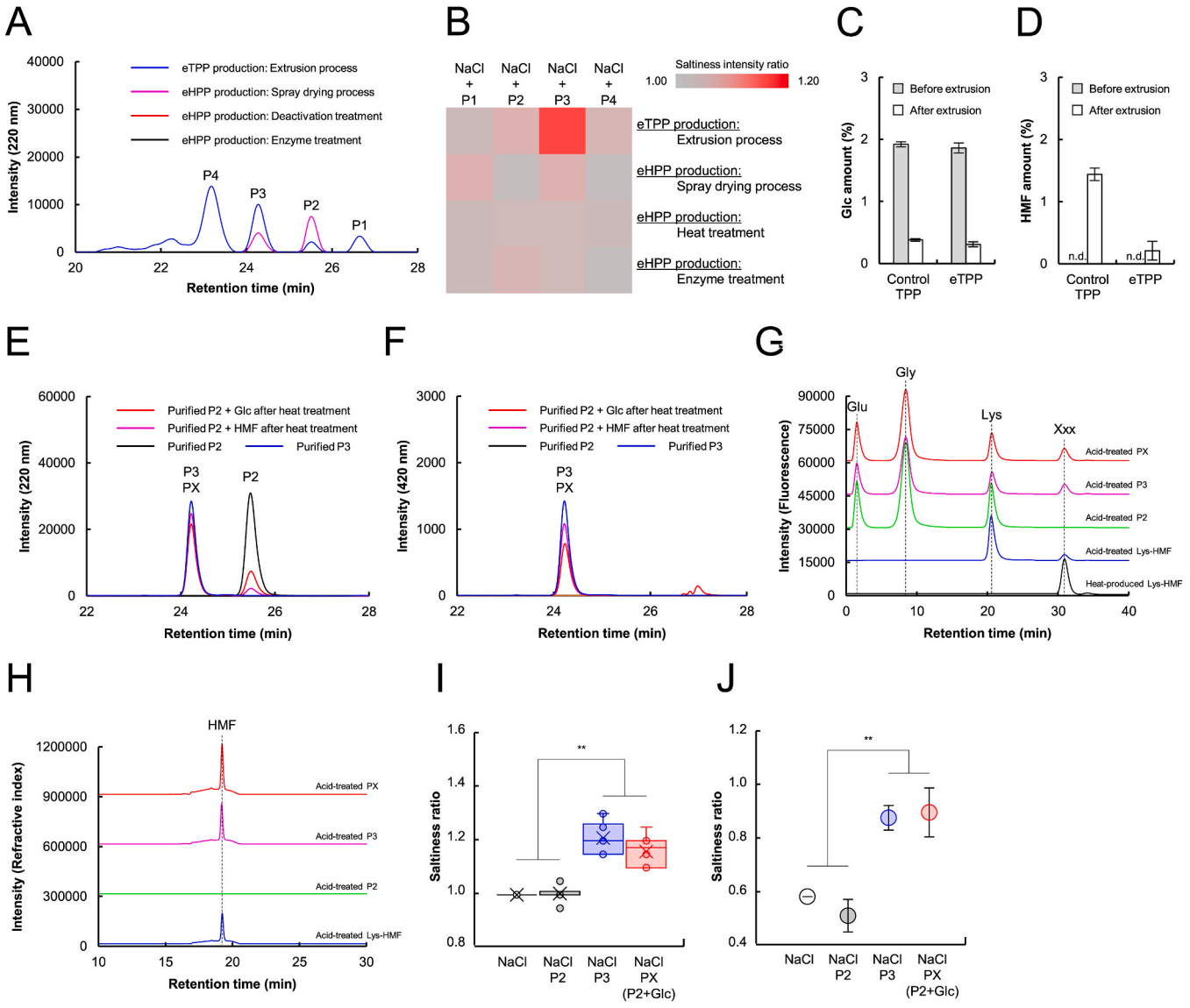

Fig. 6. Characterization of saltiness enhancement P3. (A, B) Size exclusion chromatography (A) and saltiness intensity ratio (B) of free peptides released from eHPP or eTPP at each process stage. n.d., not detected. (C, D) Quantification of the Glc (C) and HMF (D) contents in control TPP and eTPP before and after the extrusion process. (E, F) Size exclusion chromatography of the mixtures of purified P2 and Glc or HMF after heat treatment. All samples were detected at 220 nm (E) and 420 nm (F). (G, H) HPLC analysis of the acid-treated purified peptides. Amino acids (G) and HMF (H) in acid-treated hydrolysates were extracted in an aqueous layer and diethyl ether layer. (I, J) Sensory evaluations (I) and electronic tongue analysis (J) of the products of purified P2 and Glc or HMF after heat treatment compared with purified P2 and P3. The error bars represent the mean ± standard error of three independent experiments. Different letters indicate significant differences ($p < 0.05$).

eTPP, after the extrusion process the Glc content decreased, whereas the HMF content was lower than that in the control TPP. Subsequently, absorbance detection at 220 nm revealed that heat trea, tment (110 °C for 30 min) of purified P2 and Glc or HMF generated a novel peak (PX) with the same retention time as P3 (Fig. 6E). Moreover, absorbance detection at 420 nm revealed that the P3 and PX peaks had a brown color (Fig. 6F). After treatment of P2, P3, and PX with hydrochloric acid under relatively weak conditions (120 °C, 2h), Glu, Gly, and Lys were detected as amino acids present in the aqueous layer (Fig. 6G). Such a finding indicates that these peptides are composed of the same amino acids. Furthermore, a new compound, Xxx, was detected in the P3 and PX hydrolysates, exhibiting the same retention time as the heat-produced compound based on Lys and HMF. Meanwhile, HMF was detected in the diethyl ether layer of the P3 and PX hydrolysates (Fig. 6H). Such findings suggest that P3 comprised Glu-Gly-Lys-Gly and HMF was bound to Lys residues. Notably, according to the sensory evaluation and electronic tongue analysis, the purified PX based on Glc and P2 had saltiness-enhancing effects comparable to those of P3 (Fig. 6I and J). Therefore, the saltiness-enhancing P3 may be a Maillard-induced peptide based on Lys residues in Glu-Gly-Lys-Gly and HMF condensed from Glc in starch during the extrusion process.

Previous studies have shown that Maillard-reacted food-derived peptides with furan backbones can increase the saltiness enhancement effects (Zhou et al., 2021; Lan and Chen, 2024). Notably, sensory evaluation and electronic tongue analysis revealed that the brownish Maillard-reacted peptides derived from 0.25 to 1 kDa pea peptides and glucose had saltiness-enhancing effects (Yan et al., 2021). Moreover, such Maillard-reactive peptides have been reported to interact with the TRPV1 receptor to promote saltiness perception in rats (Katsumata et al., 2008). Generally, the structures of the Maillard products bound to peptides vary to different degrees, impeding the determination of their structures (Lan and Chen, 2024). Thus, the saltiness-enhancing mechanisms at the molecular level are understudied to interact with furan-backboned peptides and TRPV1 receptors. These findings suggest that protease- and glutaminase-producing Glu, Arg + Lys, and P3 (Glu-Gly-Lys-Gly containing HMF-bound Lys residues) can bind to the TMC4, ENaC, and TRPV1 receptors, thereby enhancing human saltiness perception.

3.6. *In vitro digestibility of PBMA patties*

PBMA products exhibit low digestibility due to the plant proteins and the presence of limited quantities of absorbable essential amino acids (Berrazaga et al., 2019). Therefore, we investigated the *in vitro* digestibility and amino acid profiles of the eTPP-based patties in the oral, gastric, and intestinal phases (Fig. 7). The amount of free amino nitrogen released from the eTPP-based patties was 3.9- and 1.9-fold higher than that released from the control patties during the gastric and intestinal phases, respectively (Fig. 7A). These findings indicated that the eTPP-based patties were more easily degraded by digestive proteases and had increased amino acids or peptides as absorbable nutrients. Generally, pretreated proteins that are partially hydrolyzed by enzymes are susceptible to degradation by proteases in the digestive tract (Ku et al., 2022; Zhao et al., 2019). This is because the enzymatic nick to proteins or peptides weakens the protein structure and maximizes the protein surface contact, facilitating the accessibility of the digestive enzymes to the cleavage sites (Paz-Yépez et al., 2019). The amino acid profiles of eTPP in the intestinal phase were analyzed via heat maps and clustering methods to evaluate the actual nutritional value of the eTPP-based patties (Fig. 7B). The quantities of the amino acids released from the eTPP-based patties were higher than those released from the control patties, regardless of them being non-essential or essential amino acids. Notably, the amounts of Asp, Thr, and His in the eTPP-based patties substantially increased in the intestinal phase. The absorbable type and amount of free amino acids are crucial for improving human nutrition (Delompré et al., 2019; Diepeveen et al., 2022; Dai et al., 2022). Generally, the amounts of absorbable essential amino acids in PBMA products are lower than those in animal-derived meat, creating a nutritional gap between them (McClements and Grossmann, 2022). As shown in Fig. 7B, all nine essential amino acids were increased via enzymatic treatment. These findings indicate that the eTPP-based patties may exhibit higher digestibility and bioavailability than the control patties, thereby bridging the nutritional gap between plant and animal meat products.

4. Conclusions

In this study, we aimed to improve the umami and saltiness intensity of PBMA patties using protease and glutaminase combinations while meeting clean-label requirements. Sensory evaluation revealed that the umami, kokumi, and saltiness levels of the eTPP-based patties enriched with 0.5% NaCl were significantly higher than those of the control patties enriched with 0.5% NaCl. Notably, the saltiness enhancement contributed to a 20% salt reduction in the eTPP-based patties. By screening the saltiness-enhancing amino acids and peptides released from the eTPP-based patties in artificial saliva, we revealed that the combination of Glu, Arg, Lys, and P3 enhanced the saltiness intensity of NaCl. Moreover, it was concluded that the saltiness-enhancing P3 may be a Maillard-induced peptide based on Lys residues in Glu-Gly-Lys-Gly and HMF condensed from Glc in starch during the extrusion process. Our findings suggest that eTPP produced by proteases and glutaminase could be a promising option for enhancing the umami and saltiness levels of

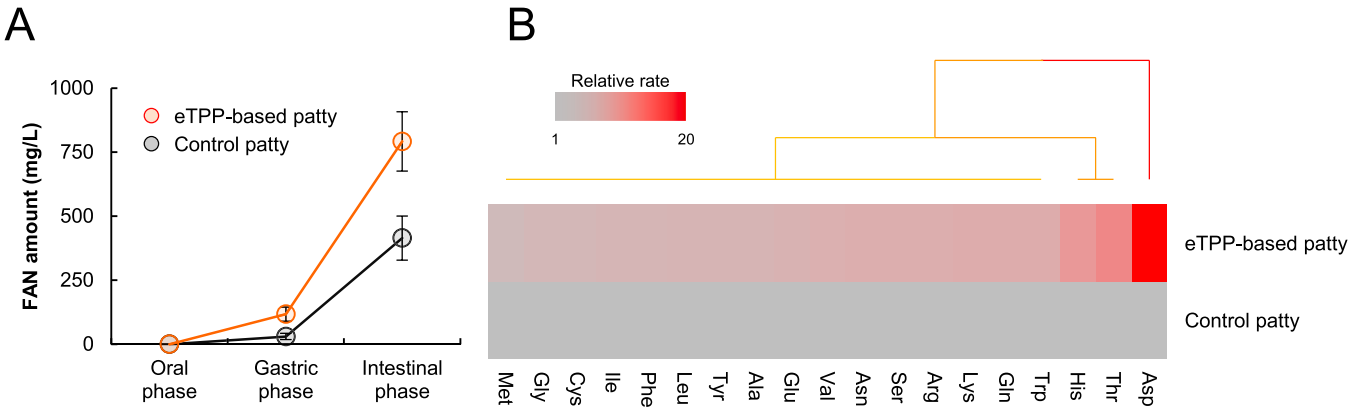

Fig. 7. *In vitro* digestion test of PBMA patties. (A) Amount of free amino nitrogen (FAN) released from the PBMA patties at the oral, gastric, and intestinal phases. The error bars represent the mean ± standard error of three independent experiments. (B) Heatmap analysis of the amino acids released from the PBMA patties at the intestinal phase.

PBMA products while meeting clean-label requirements.

CRediT authorship contribution statement

Kiyota Sakai: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Nickolas Broches:** Methodology, Investigation. **Keita Okuda:** Conceptualization, Methodology, Investigation. **Masamichi Okada:** Supervision, Project administration. **Shotaro Yamaguchi:** Supervision, Project administration.

Data availability

All data generated or analyzed during this study are included in this published article.

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Declaration of competing 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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References

Araya, M., García, S., Rengel, J., Pizarro, S., Álvarez, G., 2021. Determination of free and protein amino acid content in microalgae by HPLC-DAD with pre-column derivatization and pressure hydrolysis. Mar. Chem. 234, 103999. https://doi.org/10.1016/j.marchem.2021.103999.

Asioli, D., Aschemann-Witzel, J., Caputo, V., Vecchio, R., Annunziata, A., Næs, T., Varela, P., 2017. Making sense of the "clean label" trends: a review of consumer food choice behavior and discussion of industry implications. Food Res. Int. 99, 58–71. https://doi.org/10.1016/j.foodres.2017.07.022.

Bakhsh, A., Lee, S.J., Lee, E.Y., Sabikun, N., Hwang, Y.H., Joo, S.T., 2021. A novel approach for tuning the physicochemical, textural, and sensory characteristics of plant-based meat analogs with different levels of methylcellulose concentration. Foods 10 (3), 560. https://doi.org/10.3390/foods10030560.

Berrazaga, I., Micard, V., Gueugneau, M., Walrand, S., 2019. The role of the anabolic properties of plant-versus animal-based protein sources in supporting muscle mass maintenance: a critical review. Nutrients 11 (8), 1825. https://doi.org/10.3390/nu11081825.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., et al., 2019. INFOGEST static *in vitro* simulation of gastrointestinal food digestion. Nat. Protoc. 14 (4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1.

Chen, D., Chen, W., Wu, D., Zhang, Z., Liu, P., Li, W., Yang, Y., 2023. Saltiness enhancing peptides isolated from enzymolysis extract of *Lentinula edodes* and their taste enhancing action mechanisms. LWT–Food Sci. Technol. 188, 115430. https://doi.org/10.1016/j.lwt.2023.115430.

Chan, Q., Stamler, J., Griep, L.M.O., Daviglus, M.L., Van Horn, L., Elliott, P., 2016. An update on nutrients and blood pressure summary of INTERMAP study findings. J. Atherosclerosis Thromb. 23 (3), 276–289. https://doi.org/10.5551/jat.30000.

Cook, N.R., Appel, L.J., Whelton, P.K., 2016. Sodium intake and all-cause mortality over 20 years in the trials of hypertension prevention. J. Am. Coll. Cardiol. 68 (15), 1609–1617. https://doi.org/10.1016/j.jacc.2016.07.745.

Dai, Y., Tyl, C., 2021. A review on mechanistic aspects of individual versus combined uses of enzymes as clean label-friendly dough conditioners in breads. J. Food Sci. 86 (5), 1583–1598. https://doi.org/10.1111/1750-3841.15713.

Dai, Z., Zheng, W., Locasale, J.W., 2022. Amino acid variability, tradeoffs and optimality in human diet. Nat. Commun. 13 (1), 6683. https://doi.org/10.1038/s41467-022-34486-0.

Delompré, T., Guichard, E., Briand, L., Salles, C., 2019. Taste perception of nutrients found in nutritional supplements: a review. Nutrients 11 (9), 2050. https://doi.org/10.3390/nu11092050.

Desmond, E., 2006. Reducing salt: a challenge for the meat industry. Meat Sci. 74 (1), 188–196. https://doi.org/10.1016/j.meatsci.2006.04.014.

Diepeveen, J., Moerdijk-Poortvliet, T.C., van der Leij, F.R., 2022. Molecular insights into human taste perception and umami tastants: a review. J. Food Sci. 87 (4), 1449–1465. https://doi.org/10.1111/1750-3841.16101.

Fischer, E., Cachon, R., Cayot, N., 2020. *Pisum sativum* vs *Glycine max*, a comparative review of nutritional, physicochemical, and sensory properties for food uses. Trends Food Sci 95, 196–204. https://doi.org/10.1016/j.tifs.2019.11.021.

Flores, M., Comes, D., Gamero, A., Belloch, C., 2024. Fermentation of texturized pea protein in combination with proteases for aroma development in meat analogues. J. Agric. Food Chem. 72 (9), 4897–4905. https://doi.org/10.1021/acs.jafc.3c08432.

Hayabuchi, H., Morita, R., Ohta, M., Nanri, A., Matsumoto, H., Fujitani, S., et al., 2020. Validation of preferred salt concentration in soup based on a randomized blinded experiment in multiple regions in Japan—influence of umami (L-glutamate) on saltiness and palatability of low-salt solutions. Hypertens. Res. 43 (6), 525–533. https://doi.org/10.1038/s41440-020-0397-1.

He, J., Evans, N.M., Liu, H., Shao, S., 2020. A review of research on plant-based meat alternatives: driving forces, history, manufacturing, and consumer attitudes. Compr. Rev. Food Sci. Food Saf. 19 (5), 2639–2656. https://doi.org/10.1111/1541-4337.12610.

ISO 5492, 2008. Sensory Analysis—Vocabulary. International Organization for Standardization (ISO), Geneva, Switzerland, 2008.

ISO 8586, 2023. Sensory analysis—selection and training of sensory assessors. Warsaw, Poland: the Polish Committee for Standardization, 2023.

Jang, J., Lee, D.W., 2024. Advancements in plant based meat analogs enhancing sensory and nutritional attributes. npj Sci. Food 8 (1), 50. https://doi.org/10.1038/s41538-024-00292-9.

Katsumata, T., Nakakuki, H., Tokunaga, C., Fujii, N., Egi, M., Phan, T.H.T., et al., 2008. Effect of Maillard reacted peptides on human salt taste and the amiloride-insensitive salt taste receptor (TRPV1t). Chem. Senses 33 (7), 665–680. https://doi.org/10.1093/chemse/bjn033.

Ku, S.K., Kim, J., Kim, S.M., Yong, H.I., Kim, B.K., Choi, Y.S., 2022. Combined effects of pressure cooking and enzyme treatment to enhance the digestibility and physicochemical properties of spreadable liver sausage. Food Sci. Anim. Resour. 42 (3), 441. https://doi.org/10.5851/kosfa.2022.e14.

Kumar, P., Sharma, N., Ahmed, M.A., Verma, A.K., Umaraw, P., Mehta, N., Abubakar, A. A., Hayat, M.N., Kaka, U., Lee, S.J., Sazili, A.Q., 2022. Technological interventions in improving the functionality of proteins during processing of meat analogs. Front. Nutr. 9, 1044024. https://doi.org/10.3389/fnut.2022.1044024.

Kutzli, I., Weiss, J., Gibis, M., 2021. Glycation of plant proteins via maillard reaction: reaction chemistry, technofunctional properties, and potential food application. Foods 10 (2), 376. https://doi.org/10.3390/foods10020376.

Lan, H., Chen, L., 2024. Salt reduction strategies and enhanced saltiness perception mechanisms for prepared dishes: a review. Food Safety and Health 2 (3), 362–379. https://doi.org/10.1002/fsh3.12055.

Lao, Y., Ye, Q., Wang, Y., Selomulya, C., 2024. Modulating digestibility and mitigating beany flavor of pea protein. Food Rev. Int. 40 (9), 3129–3158. https://doi.org/10.1080/87559129.2024.2346329.

Le, B., Yu, B., Amin, M.S., Liu, R., Zhang, N., Soladoye, O.P., et al., 2022. Salt taste receptors and associated salty/salt taste-enhancing peptides: a comprehensive review of structure and function. Trends Food Sci. Technol. 129, 657–666. https://doi.org/10.1016/j.tifs.2022.11.014.

Lee, J.S., Oh, H., Choi, I., Yoon, C.S., Han, J., 2022. Physico-chemical characteristics of rice protein-based novel textured vegetable proteins as meat analogues produced by low-moisture extrusion cooking technology. LWT (Lebensm.-Wiss. & Technol.) 157, 113056. https://doi.org/10.1016/j.lwt.2021.113056.

Lei, F., Hu, C., Zhang, N., He, D., 2019. The specificity of an aminopeptidase affects its performance in hydrolyzing peanut protein isolate and zein. LWT–Food Sci. Technol. 102, 37–44. https://doi.org/10.1016/j.lwt.2018.10.041.

Li, H., Zheng, R., Zuo, F., Qian, C., Yao, Z., Dong, R., et al., 2022. Influence of proteolysis on the binding capacity of flavor compounds to myofibrillar proteins. Foods 11 (6), 891. https://doi.org/10.3390/foods11060891.

Martínez, Y.B., Ferreira, F.V., Musumeci, M.A., 2022. l-Glutamine-, peptidyl-and protein-glutaminases: structural features and applications in the food industry. World J. Microbiol. Biotechnol. 38 (11), 204. https://doi.org/10.1007/s11274-022-03391-5.

McClements, D.J., Grossmann, L., 2021. The science of plant-based foods: constructing next-generation meat, fish, milk, and egg analogs. Compr. Rev. Food Sci. Food Saf. 20 (4), 4049–4100. https://doi.org/10.1111/1541-4337.12771.

McClements, D.J., Grossmann, L., 2022. Next-Generation Plant-Based Foods: Design, Production, and Properties. Springer, New York. https://doi.org/10.1007/978-3-030-96764-2.

Paz-Yépez, C., Peinado, I., Heredia, A., Andrés, A., 2019. Influence of particle size and intestinal conditions on *in vitro* lipid and protein digestibility of walnuts and peanuts. Food Res. Int. 119, 951–959. https://doi.org/10.1016/j.foodres.2018.11.014.

Peñaranda, I., Garrido, M.D., García-Segovia, P., Martínez-Monzó, J., Igual, M., 2023. Enriched pea protein texturing: physicochemical characteristics and application as a substitute for meat in hamburgers. Foods 12 (6), 1303. https://doi.org/10.3390/foods12061303.

Plattner, B.J., Hong, S., Li, Y., Talavera, M.J., Dogan, H., Plattner, B.S., Alavi, S., 2024. Use of pea proteins in high-moisture meat analogs: physicochemical properties of raw formulations and their texturization using extrusion. Foods 13 (8), 1195. https://doi.org/10.3390/foods13081195.

Saffarionpour, S., 2024. Off-flavors in pulses and grain legumes and processing approaches for controlling flavor-plant protein interaction: application prospects in plant-based alternative foods. Food Bioprocess Technol. 17 (5), 1141–1182. https://doi.org/10.1007/s11947-023-03148-4.

Sakai, K., Okada, M., Yamaguchi, S., 2022. Decolorization and detoxication of plant-based proteins using hydrogen peroxide and catalase. Sci. Rep. 12 (1), 22432. https://doi.org/10.1038/s41598-022-26883-8.

Sakai, K., Okada, M., Yamaguchi, S., 2023. Protein-glutaminase improves water-/oil-holding capacity and beany off-flavor profiles of plant-based meat analogs. PLoS One 18 (12), e0294637. https://doi.org/10.1371/journal.pone.0294637.

Sakai, K., 2024. Functional properties of meat analog products consisting of plant-derived proteins. In: Handbook of Plant-Based Meat Analogs. Academic Press, pp. 347–375. https://doi.org/10.1016/B978-0-443-21846-0.00007-1.

Sakai, K., Okada, M., Yamaguchi, S., 2024b. Enhanced textural properties of plant-based patties treated using crosslinking-catalyzed enzymes compared with those of beef patties. Food Sci. Technol. Res. 30 (4), 467–477. https://doi.org/10.3136/fstr.FSTR-D-24-00014.

Sakai, K., Okada, M., Yamaguchi, S., 2024c. Umami and saltiness enhancements of vegetable soup by enzyme-produced glutamic acid and branched-chain amino acids. Front. Nutr. 11, 1436113. https://doi.org/10.3389/fnut.2024.1436113.

Sakai, K., Okada, M., Yamaguchi, S., 2025. Glutamic acid production methods by protease and protein-glutaminase for plant-based meat analog patties. Food Sci. Technol. Res. 31 (3). https://doi.org/10.3136/fstr.FSTR-D-24-00222.

Scarton, M., Ganancio, J.R.C., de Avelar, M.H.M., Clerici, M.T.P.S., Steel, C.J., 2021. Lime juice and enzymes in clean label pan bread: baking quality and preservative effect. J. Food Sci. Technol. 58, 1819–1828. https://doi.org/10.1007/s13197-020-04693-y.

Shen, D.Y., Pan, F., Yang, Z.C., Song, H.L., Zou, T.T., Xiong, J., Li, K., Li, P., Hu, N., Xue, D.D., 2022. Identification of novel saltiness-enhancing peptides from yeast extract and their mechanism of action for transmembrane channel-like 4 (TMC4) protein through experimental and integrated computational modeling. Food Chem. 388, 132993. https://doi.org/10.1016/j.foodchem.2022.132993.

Shi, X., Hao, Z., Wang, R., Chen, Z., Zuo, F., Wan, Y., Guo, S., 2022. Changes of hexanal content in fermented soymilk: induced by lactic acid bacterial fermentation and thermal treatment. J. Food Process. Preserv. 46 (5), e16555. https://doi.org/10.1111/jfpp.16555.

Song, P., Zhang, X., Wang, S., Xu, W., Wang, F., Fu, R., Wei, F., 2023. Microbial proteases and their applications. Front. Microbiol. 14, 1236368. https://doi.org/10.3389/fmicb.2023.1236368.

Sun, X., Zheng, J., Liu, B., Huang, Z., Chen, F., 2022a. Characteristics of the enzyme-induced release of bitter peptides from wheat gluten hydrolysates. Front. Nutr. 9, 1022257. https://doi.org/10.3389/fnut.2022.1022257.

Sun, X., Zhong, K., Zhang, D., Shi, B., Wang, H., Shi, J., et al., 2022b. The enhancement of the perception of saltiness by umami sensation elicited by flavor enhancers in salt solutions. Food Res. Int. 157, 111287. https://doi.org/10.1016/j.foodres.2022.111287.

Tang, C.H., Wang, X.S., Yang, X.Q., 2009. Enzymatic hydrolysis of hemp (*Cannabis sativa* L.) protein isolate by various proteases and antioxidant properties of the resulting hydrolysates. Food Chem. 114 (4), 1484–1490. https://doi.org/10.1016/j.foodchem.2008.11.049.

Tomé, D., 2021. Yeast extracts: nutritional and flavoring food ingredients. ACS Food Sci. Technol 1 (4), 487–494. https://doi.org/10.1021/acsfoodscitech.0c00131.

Trindler, C., Kopf-Bolanz, K.A., Denkel, C., 2022. Aroma of peas, its constituents and reduction strategies–Effects from breeding to processing. Food Chem. 376, 131892. https://doi.org/10.1016/j.foodchem.2021.131892.

Wang, H., Xu, J., Liu, Q., Chen, Q., Sun, F., Kong, B., 2022. Interaction between protease from Staphylococcus epidermidis and pork myofibrillar protein: flavor and molecular simulation. Food Chem. 386, 132830. https://doi.org/10.1016/j.foodchem.2022.132830.

United Nations Department of Economic and Social Affairs, 2024. World population prospects 2024: summary of results. Geneva. https://desapublications.un.org/file/20847/download. (Accessed 3 December 2024).

Wang, K., Arntfield, S.D., 2015. Binding of selected volatile flavour mixture to salt-extracted canola and pea proteins and effect of heat treatment on flavour binding. Food Hydrocoll. 43, 410–417. https://doi.org/10.1016/j.foodhyd.2014.06.011.

Wang, K., Arntfield, S.D., 2017. Effect of protein-flavour binding on flavour delivery and protein functional properties: a special emphasis on plant-based proteins. Flavour Fragrance J. 32 (2), 92–101. https://doi.org/10.1002/ffj.3365.

Wang, W., Zhou, X., Liu, Y., 2020. Characterization and evaluation of umami taste: a review. Trends Anal. Chem. 127, 115876. https://doi.org/10.1016/j.trac.2020.115876.

Wang, Z., Cheng, Y., Muhoza, B., Sun, M., Feng, T., Yao, L., et al., 2024. Discovery of peptides with saltiness-enhancing effects in enzymatic hydrolyzed *Agaricus bisporus* protein and evaluation of their salt-reduction property. Food Res. Int. 177, 113917. https://doi.org/10.1016/j.foodres.2023.113917.

Wu, H., Sakai, K., Zhang, J., McClements, D.J., 2024. Plant-based meat analogs: color challenges and coloring agents. Food, Nutrition and Health 1 (1), 4. https://doi.org/10.1007/s44403-024-00005-w.

Xie, X., Dang, Y., Pan, D., Sun, Y., Zhou, C., He, J., Gao, X., 2023. The enhancement and mechanism of the perception of saltiness by umami peptide from *Ruditapes philippinarum* and ham. Food Chem. 405, 134886. https://doi.org/10.1016/j.foodchem.2022.134886.

Xu, J.J., Elkaddi, N., Garcia-Blanco, A., Spielman, A.I., Bachmanov, A.A., Chung, H.Y., Ozdener, M.H., 2017. Arginyl dipeptides increase the frequency of NaCl-elicited responses via epithelial sodium channel alpha and delta subunits in cultured human fungiform taste papillae cells. Sci. Rep. 7 (1), 7483. https://doi.org/10.1038/s41598-017-07756-x.

Yamamoto, T., Inui-Yamamoto, C., 2023. The flavor-enhancing action of glutamate and its mechanism involving the notion of kokumi. npj Sci. Food 7 (1), 3. https://doi.org/10.1038/s41538-023-00178-2.

Yan, F., Cui, H., Zhang, Q., Hayat, K., Yu, J., Hussain, S., et al., 2021. Small peptides hydrolyzed from pea protein and their Maillard reaction products as taste modifiers: saltiness, umami, and kokumi enhancement. Food Bioprocess Technol. 14, 1132–1141. https://doi.org/10.1007/s11947-021-02630-1.

Zhang, J., Sun-Waterhouse, D., Su, G., Zhao, M., 2019. New insight into umami receptor, umami/umami-enhancing peptides and their derivatives: a review. Trends Food Sci. Technol. 88, 429–438. https://doi.org/10.1016/j.tifs.2019.04.008.

Zhang, J., Kang, D., Zhang, W., Lorenzo, J.M., 2021. Recent advantage of interactions of protein-flavor in foods: perspective of theoretical models, protein properties and extrinsic factors. Trends Food Sci. Technol. 111, 405–425. https://doi.org/10.1016/j.tifs.2021.02.060.

Zhao, D., Xu, Y., Gu, T., Wang, H., Yin, Y., Sheng, B., et al., 2019. Peptidomic investigation of the interplay between enzymatic tenderization and the digestibility of beef semimembranosus proteins. J. Agric. Food Chem. 68 (4), 1136–1146. https://doi.org/10.1021/acs.jafc.9b06618.

Zhou, X., Cui, H., Zhang, Q., Hayat, K., Yu, J., Hussain, S., et al., 2021. Taste improvement of Maillard reaction intermediates derived from enzymatic hydrolysates of pea protein. Food Res. Int. 140, 109985. https://doi.org/10.1016/j.foodres.2020.109985.