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Microbial-dependent variations in umami compounds during fermentation of prawn by-products

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This study investigated the effects of microbial fermentation on the umami taste components of prawn by-products. Six microorganisms — *Aspergillus oryzae*, *Rhizopus oligosporus*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Lactiplantibacillus plantarum* and *Latilactobacillus sakei* — were used to ferment prawn by-product juice. The resulting broths were analyzed for umami-related compounds, including free amino acids and flavor nucleotides. Taste activity values (TAVs) and equivalent umami concentrations (EUCs) were calculated to assess umami intensity. Fermentation enhanced acidity and significantly increased the concentrations of umami-active compounds. The EUC values increased markedly from 0.23 g monosodium glutamate (MSG)/100 g at day 0 to 6.36 g MSG/100 g on day 3 in the samples fermented with *R. oligosporus*. Glutamic acid was identified as the dominant umami taste compound and its TAV exceeded 1 in fermented samples, confirming a perceptible umami contribution. Among the tested strains, *R. oligosporus* produced the most pronounced umami profile, indicating its potential as an effective starter culture for transforming prawn by-products into value-added, umami-rich ingredients.

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Sustainability spotlight

This research introduces a sustainable pathway for the food circular economy by valorizing prawn side-streams, which are a common waste stream from seafood processing, into a high-value food ingredient using fermentation. By reducing reliance on non-renewable resources and transforming waste into functional products, this innovation supports circular economy principles, promotes healthier diets through natural flavors, and significantly reduces food waste, directly addressing UN SDGs 3, 9, and 12 for a more resilient food system.

1. Introduction

Prawn is one of the most widely traded seafood commodities globally, with international trade volumes exceeding 3.7 million tonnes in recent years.[1] Due to their high perishability, fresh prawns are usually processed at low temperatures upon harvest to extend their shelf life. However, such processing generates large quantities of by-products, namely the head, shell, and tail, which can account for approximately 45–60% of the total prawn weight, depending on the species and processing method.[2] The global prawn market, valued at approximately USD 74.7 billion in 2025 and projected to reach USD 106.1 billion by 2034, continues to expand, driven largely by the dominance of the *Litopenaeus vannamei* species, which contributes over 70% of global production.[3] This growth is expected to further increase the volume of processing residues, which is currently estimated at around 3.8 million tonnes annually.[4] The accumulation of these by-products presents both environmental and economic challenges, highlighting the urgent need for sustainable valorization strategies. These by-products, particularly prawn heads, are rich in free amino acids and nucleotides, making them well-suited for the production of flavor-enhancing ingredients.[5]

Fermentation, a traditional preservation and processing method, has been widely recognized for improving the sensory attributes of foods, particularly flavor and texture. Microorganisms play a pivotal role in this process; through their metabolic activities, they transform food components, imparting unique sensory characteristics and potential health benefits.[6] Fermentation has also been applied to seafood products, such as fish sauces, prawn pastes, and fermented fish, not only for preservation but also to enhance flavor and palatability.[7]

Voidarou *et al.*[8] classified food fermentation processes into three major types, depending on the dominant microorganism and its metabolic activities: lactic acid fermentation, fungal fermentation and alkaline fermentation. The filamentous fungus *Aspergillus oryzae*, used in soy sauce fermentation, contributes to its characteristic umami flavor through the production of amino acids, nucleotides, and organic acids.[9] Similarly, *Rhizopus oligosporus*, utilized in the production of tempeh, enhances umami intensity through the breakdown of

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soybean proteins to amino acids.[10] Yeasts, in particular *Saccharomyces cerevisiae*, are commonly employed in the production of fermented beverages like beer and wine, generating esters, organic acids, and other aroma-active products that define the sensory profiles of these fermented beverages.[11] In alkaline fermentation, *Bacillus subtilis* has also been associated with improved sensory quality in pulses and teas by generating peptides and amino acids and reducing undesirable flavor and odor compounds, such as hexanal, a compound responsible for 'grassy' and 'beany' off-flavors.[12,13] Among lactic acid bacteria (LAB), *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) has been widely used due to its versatility and has been found to enhance the umami flavor through the release of amino acids.[14] *Latilactobacillus sakei* (formerly *Lactobacillus sakei*), another lactic acid bacterium, contributes to flavor development in a diverse range of substrates, such as rice in sake,[15] cabbage in kimchi[16] and meat in sausages,[17,18] through the production of fermentation metabolites.

Flavor is a critical determinant of consumer preference and an important indicator of product quality.[19] It encompasses both taste and odor, which arise from the stimulation of gustatory and olfactory receptors by non-volatile and volatile compounds, respectively.[20] Umami, one of the five basic taste sensations, plays a critical role in defining the palatability of seafood. In prawns, this taste primarily arises from synergistic interactions among free amino acids, nucleotides, inorganic ions, and organic acids.[21]

Recent research on prawn by-products has mainly focused on recovering chitin, proteins, peptides, carotenoids, and antioxidants from solid waste such as heads, shells, and tails, often using enzymatic hydrolysis or microbial fermentation.[22–27] In industry, prawn waste is typically pressed into liquid and solid fractions to reduce processing energy for the solid fraction. The liquid fraction, accounting for approximately 70% (w/w) of total waste, is often discarded into wastewater streams, increasing treatment costs and resulting in the loss of valuable nutrients, while the solid fraction is utilized for prawn shell meal or chitin and chitosan production.[28] To address this inefficiency, Nguyen *et al.*[28] explored the valorization of prawn liquid waste *via* enzymatic hydrolysis, producing nutrient-rich protein hydrolysates with potential applications in aquaculture. Nevertheless, the potential of fermentation to enhance flavor and umami in prawn by-product juice remains largely unexplored, despite evidence that microbial processes can generate taste-active compounds such as free amino acids and nucleotides in solid substrates.[29]

As a result, there is a significant gap in the understanding of how microbial fermentation will impact the umami characteristics of prawn by-product juice. Hence, to address this knowledge gap, this present study explores the fermentation of prawn by-product juice using six microorganisms, namely *A. oryzae*, *R. oligosporus*, *S. cerevisiae*, *B. subtilis*, *L. plantarum* and *L. sakei*. The resulting fermented products were analyzed for flavor-related compounds, specifically free amino acids and flavor nucleotides, to identify the most effective microorganism for enhancing umami intensity. This study focused on umami-contributing compounds; however, free amino acids (FAAs) with sweet or bitter attributes are also reported to provide a more comprehensive sensory profile. The findings are expected to provide a theoretical foundation for the valorization of prawn by-products through the development of novel umami-rich fermented ingredients. The growing emphasis on sustainability and the adoption of green technologies to manage food waste further underscore the significance of this work, which aims to advance a zero-waste approach while creating a natural prawn-based flavoring with health benefits.

2. Methods and materials

2.1. Materials and reagents

LC-MS grade acetonitrile (ACN) and formic acid were purchased from Fisher Scientific. Adenosine-5′monophosphate disodium salt (≥99.0%, HPLC), guanosine-5′-monophosphate disodium salt hydrate (from yeast, ≥99%), and inosine-5′-monophosphate disodium salt hydrate (≥99.0%, HPLC), amino acid standards (AAS18), HPLC-grade hexane, and LiChropur-grade reagents for LC-MS analysis, including ammonium acetate, ammonium formate, and 25% ammonia solution were purchased from Sigma-Aldrich (St. Louis, MO, USA). All water used in this research project was ultrapure (Type 1) and obtained from a LaboStar PRO TWF UV ultrapure water system (Evoqua Water Technologies, Günzburg, Germany).

Individual nucleotide stock solutions (10 mM) were prepared in ultrapure water from the commercial standards without further purification and stored at −20 °C in the dark. Working standard solutions were freshly prepared daily. The amino acid standard mixture was also prepared freshly on the day of analysis.

2.2. Sample preparation

Fresh prawns were purchased from a local supermarket (NTUC FairPrice, Singapore). The samples were marketed as Pasar Vannamei Prawn (*L. vannamei*), whole prawn with head and shell, with a size grade of 'L' (body length: 15.5 ± 0.4 cm; weight: 23.8 ± 1.05 g). The samples were transported to the laboratory under chilled conditions and were stored at −20 °C upon arrival until further processing. The prawn heads, shells and tails were manually separated from the meat and rinsed three times with ultrapure water. The by-products were blended with water at a 1 : 1 (w/v) ratio using a Ninja Foodi Power Blender (Extract Function). The homogenate was filtered through a 100-mesh nylon food sieve to obtain prawn juice, which was subsequently lyophilized and stored at −20 °C until further processing.

Prior to fermentation, the lyophilized prawn juice powder was reconstituted with ultrapure water to a concentration of 5% (w/v) and pasteurized in a water bath at 90 °C for 30 min. The absence of viable microorganisms in the reconstituted pasteurized prawn juice was confirmed by plating on a variety of selective and non-selective agar, including potato dextrose agar, Mueller–Hinton agar, Lysogeny Broth agar, plate count agar, Sabouraud dextrose agar with chloramphenicol, and De Man, Rogosa, and Sharpe Agar (data not shown).

2.3. Microorganisms and culture conditions

The microbial strains used in this study were *A. oryzae* DSM 1147, *R. oligosporus* DSM 1964, *S. cerevisiae* BY4741, *B. subtilis* ATCC 6051, *L. plantarum* ATCC 14917, and *L. sakei* subsp. *sakei* ATCC 15521. Each microorganism was maintained and activated in its corresponding culture medium prior to inoculation (Table S1). The cells or spores of the activated microorganisms were collected by centrifugation at 8000×*g* for 10 min at 4 °C and washed twice with sterile phosphate buffer (pH 7.0). The resulting cell or spore suspension was used as the inoculum for fermentation experiments.

2.4. Fermentation of prawn juice

The reconstituted pasteurized prawn juice substrate was supplemented with 2% (w/v) glucose to support microbial growth. Fermentation was initiated by inoculating with 1% (v/v) of the cell (*S. cerevisiae*, *B. subtilis L. plantarum*, and *L. sakei*) (approx. 10^8 CFU mL^{-1}) or spore suspension (*A. oryzae* and *R. oligosporus*) (approx. 10^7 spores per mL).

Fermentations were conducted for 5 days under the optimal temperature conditions for each microorganism. The samples were collected on days 1, 2, 3, and 5 to monitor the development of flavor compounds. Following sampling, the pH of the fermentation broths was measured with a calibrated pH meter (FiveEasy F20, Mettler-Toledo, Greifensee, Switzerland), and the fermentation broths were stored at −20 °C until further analysis.

2.5. Determination of free amino acids

The samples were diluted with acetonitrile: water (1 : 1, v/v) containing 0.1% formic acid. The mixture was centrifuged at 12 000×*g* for 10 min at 4 °C. The supernatant was transferred into LC vials for LC-MS analysis. Working standards (0.5–20 μM) were used to construct an external calibration curve (R^2 > 0.99) for detection and quantification of the free amino acids.

Chromatographic separation was performed using an Agilent 1290 Infinity II Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a multi-sampler module and a high-speed binary pump. An Agilent Zorbax HILIC Plus column (3.0 × 100 mm, 1.8 μm) coupled with a HILIC-PLUS guard column (3.0 × 5 mm, 1.8 μm) was used. 20 mM ammonium formate in water with 0.1% formic acid was used as mobile phase A, and 20 mM ammonium formate in acetonitrile/water (9 : 1, v/v) with 0.1% formic acid was used as mobile phase B. The flow rate was maintained at 0.4 mL min^{-1}, column temperature at 30 °C and injection volume at 5 μL. The gradient program was initiated at 100% B, followed by a gradient to 89% at 5 min, 88% B at 7 min, and 75% B by 11 min, 70% B by 15 min. Initial conditions of 100% B were re-established at 17 min which was held until 20 min for re-equilibration. Detection was performed using an Agilent 6456 Q-TOF mass spectrometer with an Agilent Jet Stream Dual electrospray ionization (AJS-Dual ESI) interface operating in positive ionization mode. Source conditions were as follows: gas temperature, 150 °C; drying gas flow, 10 L min^{-1}; nebulizer pressure, 20 psi; sheath gas temperature, 400 °C; sheath gas flow, 12 L min^{-1}; capillary voltage (V_{cap}), 2000 V; nozzle voltage, 0 V; fragmentor voltage, 80 V; skimmer, 65 V; and octupole RF Vpp, 750 V. Data were acquired over *m*/*z* 50–1200 at 2.0 spectra per sec.

2.6. Determination of free nucleotides

Nucleotides were extracted using a combination of solvent precipitation and liquid–liquid extraction (LLE). Ice-cold solutions of 10 mM ammonium acetate were prepared (i) in water (pH 9) and (ii) in acetonitrile/water (9 : 1, v/v, pH 9). These were added to the samples in a volumetric ratio of 1 : 2 : 1 (aqueous buffer: organic phase: sample), followed by 30 s vortex mixing, and kept at −30 °C for 15 min to precipitate proteins. The samples were centrifuged (24 446×*g*, 4 °C, 15 min) and the supernatant was mixed with hexane (1 : 3, v/v),[30] followed by centrifugation under the same conditions. The aqueous layer was subjected to LC-QTOF analysis. Working standards (0.5–50 μM) were used to construct an external calibration curve (R^2 > 0.99) for detection and quantification of the free nucleotides.

Separation of AMP, IMP, and GMP was carried out on an Agilent InfinityLab Poroshell 120 HILIC-Z column (100 × 2.1 mm, 2.7 μm) coupled with a HILIC-Z guard column (5 × 2.1 mm, 2.7 μm) maintained at 30 °C. 10 mM ammonium acetate in water, pH 9, was used as mobile phase A and 10 mM ammonium acetate in acetonitrile/water (9 : 1, v/v), pH 9, adjusted by ammonia solution 25% was used as mobile phase B. The elution program was as follows: 90% B initially, decreasing linearly to 50% B over 12 min (held for 1 min), then returning to 90% within 2 min, and equilibrating for 5 min (total run time 20 min). Injection volume was 10 μL.

The QTOF-MS parameters followed those reported by Pastor-Belda *et al.*[31] with modifications: negative ionization mode; nebulizer gas pressure, 40 psi; drying gas, 13 L min^{-1} at 200 °C; sheath gas, 12 L min^{-1} at 300 °C; capillary voltage, 2500 V; nozzle voltage, 100 V; fragmentor voltage, 350 V; skimmer voltage, 65 V and octupole RF Vpp, 750 V. Data were acquired from *m*/*z* 100–1200 at 3 spectra per sec.

2.7. Evaluation of umami taste contribution

The taste activity value (TAV) is used to evaluate the relative contribution of an individual compound to the overall taste perception. It is defined as the ratio of a compound's concentration in the sample to its corresponding taste threshold, indicates that the compound contributes perceptibly to the overall taste profile when exceeding 1:[32]

$$\mathrm{TAV} = \frac{C_1}{C_2} \quad (1)$$

where C_1 is the concentration of the taste compound and C_2 is its threshold concentration.

The equivalent umami concentration (EUC) quantifies the synergistic umami intensity effect of specific amino acids, namely L-aspartic acid (Asp) and L-glutamic acid (Glu), in combination with nucleotides such as inosine monophosphate (IMP), guanosine monophosphate (GMP), and adenosine

monophosphate (AMP). The EUC expresses the umami intensity as the equivalent concentration of monosodium glutamate (MSG) per 100 g, was calculated as follows:

$$\mathrm{EUC} = \sum_i a_i b_i + 1218 \times \left(\sum_i a_i b_i\right) \times \left(\sum_j a_j b_j\right) \quad (2)$$

where a_i and a_j represent the concentrations (g/100 g) of umami amino acids (L-Asp and L-Glu) and nucleotides (AMP, GMP, and IMP), respectively, and b_i denotes the relative umami concentration (RUC) of amino acids (L-Asp = 0.077, L-Glu = 1); b_j represents the RUC of nucleotides (AMP = 0.18, GMP = 2.3, IMP = 1); and 1218 is the synergistic constant reflecting the interaction between umami amino acids and nucleotides.[33]

2.8. Data processing and statistical analysis

LC-QTOF data were processed using Agilent MassHunter Quantitative Analysis software (Q-TOF, Quant-My-Way, version 10.1). Compounds were identified based on the retention time and m/z values and quantified using external calibration curves prepared from standard solutions of known concentrations analyzed under identical conditions. Data visualization and statistical analysis were performed using Microsoft Excel 2016 (Microsoft Ltd, WA, USA) and OriginPro 2024 (OriginLab Corporation, MA, USA). One-way ANOVA and two-way ANOVA followed by Tukey's test were applied, with statistical significance set at $\alpha = 0.05$. The results are reported as mean ± standard deviation (S.D.).

3. Results and discussion

3.1. Changes in the pH during fermentation

Fig. 1 illustrates the pH changes of the prawn by-product juice fermented with different microbial strains. The pH of the prawn by-product juice decreased progressively during fermentation with all tested microorganisms, consistent with acidification associated with microbial activity.[22] The most pronounced acidification occurred in the samples fermented with *L. plantarum* followed by *L. sakei*, both of which are lactic acid bacteria known for their efficient carbohydrate utilization and lactic acid formation.[34] The reduction in pH may contribute to the suppression of undesirable microorganisms and promote proteolysis, thereby enhancing the release of flavor-related compounds.[35] In contrast, fermentation with *S. cerevisiae*, *A. oryzae*, *R. oligosporus* and *B. subtilis*, resulted in more moderate pH reduction. This milder acidification may be attributed to the high protein and amino-nitrogen content of prawn by-products, which provide buffering capacity against sharp pH changes.[36] Also, it is observed that there is slight rebound in the pH in the fermentation of *A. oryzae*, *S. cerevisiae*, *B. subtilis* and *L. plantarum*. This can be explained by the accumulation of alkaline metabolites during protein degradation in the later stages of fermentation, which may act as a buffer against organic acids, metabolites typically produced in fermentation.[37] Overall, the observed pH changes indicate that fermentation modifies the biochemical environment of prawn by-products, thereby facilitating the development of distinct flavor profiles through microorganism-specific metabolic activities.

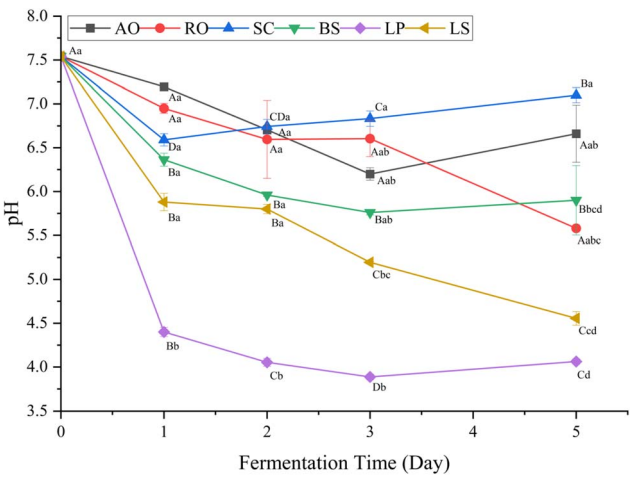

Fig. 1 Changes in the pH of the prawn by-product juice fermented with different microbial strains throughout the fermentation period. Lowercase superscript letters represent significantly different values between microbial strains within the same day; uppercase superscript letters represent significantly different values between fermentation days of a specific microbial strain (Tukey test, $p < 0.05$).

3.2. Effects of fermentation with different microorganisms on free amino acids

Free amino acids (FAAs) play an important role in flavor development, contributing to the overall sensory characteristics of fermented products. The FAA profile influences taste perception, contributing to umami (glutamic acid and aspartic acid), sweetness (serine, threonine, glycine, alanine and proline), and bitterness (valine, methionine, isoleucine, leucine, phenylalanine, histidine and arginine).[38] The content of 17 free amino acids of the fermented prawn by-product juice can be found in Table S2.

The changes in FAA concentrations of the prawn by-product juice fermented with different microbial strains throughout the fermentation period are shown in Fig. 2. The total FAA content decreased to its lowest on day 2, suggesting rapid microbial uptake or utilization of the available nitrogen source during the early fermentation stage. A similar reduction in FAAs has been reported in other fermentation systems, such as Pu-erh ripened tea[39] and dried longan fermentation.[40] The subsequent increase of total FAA content on day 3 likely resulted from enhanced proteolytic activity and peptide hydrolysis during fermentation, indicating active protein degradation by microbial enzymes. The slight decrease observed on day 5 could be attributed to the continued metabolism of amino acids as energy sources or as precursors for secondary metabolite synthesis.[41]

Among the tested microorganisms, *L. plantarum* and *R. oligosporus* produced the highest total FAA concentration after five days of fermentation, suggesting their strong proteolytic capacity under the fermentation conditions employed (Table 2). Notably, the concentrations of umami-related amino acids, particularly glutamic acid (Glu) and aspartic acid (Asp), were

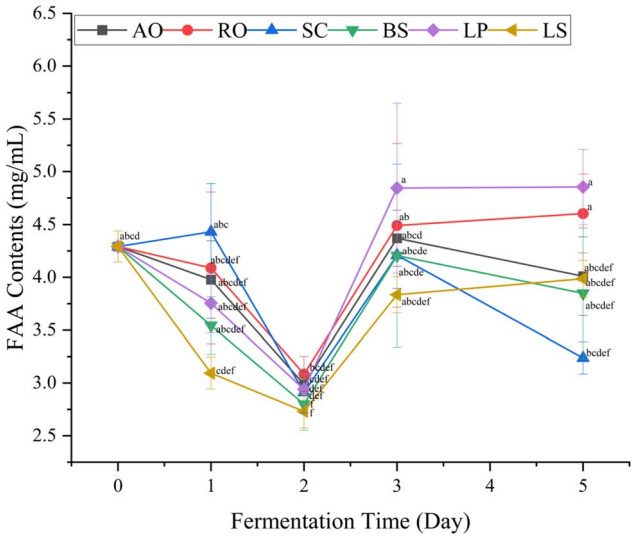

Fig. 2 Changes in free amino acid (FAA) contents of the prawn by-product juice fermented with different microbial strains throughout the fermentation period. Lowercase superscript letters represent significantly different values (two-way ANOVA, Tukey test, $p < 0.05$).

significantly elevated in all fermentations after day 3 (Fig. 3). This trend is likely due to the high abundance of these amino acids in *L. vannamei* head and shell proteins, coupled with microbial proteolytic activity during fermentation, which progressively hydrolyzed proteins into free amino acids.[42,43] This is in agreement with the findings by Lim *et al.*,[44] where fermentation led to a significant accumulation of Glu and Asp in shrimp paste. These amino acids are key contributors to savory taste perception and serve as precursors for umami enhancement.[45] The sweet- and bitter-tasting amino acids follow the trend similar to that of total amino acids (Table 1).

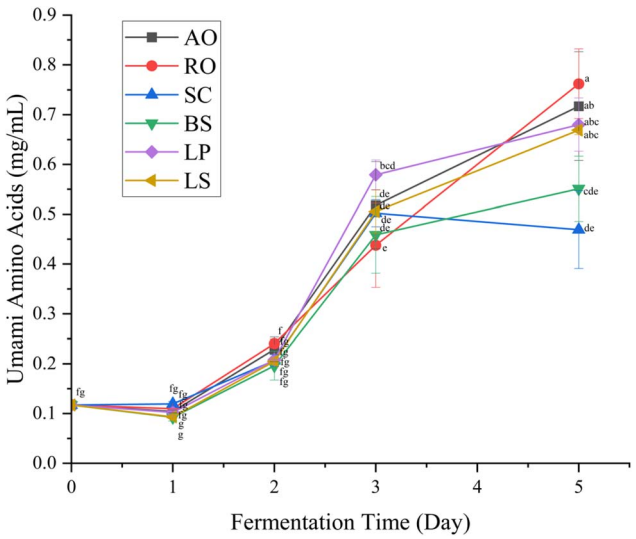

Fig. 3 Changes in umami amino acid concentrations of the prawn by-product juice fermented with different microbial strains throughout the fermentation period. Lowercase superscript letters represent significantly different values (two-way ANOVA, Tukey test, $p < 0.05$).

Different microbial strains produce distinct types of proteases, leading to variations in protein breakdown: for instance, bacteria such as *Bacillus* mainly produce alkaline proteases, while molds like *Aspergillus* and *Rhizopus* predominantly produce neutral and acid proteases.[46–48] Microbial proteases can be further classified by their catalytic type, such as aspartic endoproteases, cysteine/thiol endoproteases, metalloendoproteases, serine endoproteases, and glutamic acid and threonine endoproteases.[49] Once proteins are hydrolyzed, the resulting amino acids can undergo further microbial metabolism through processes such as transamination, deamination, and decarboxylation, generating secondary metabolites.[50] This strain-specific diversity in protease type, abundance, and activity helps explain why different microbes produce varying levels of umami amino acids.

3.3. Changes in free nucleotide contents during fermentation

Nucleotides are important flavor-enhancing compounds commonly found in aquatic products, contributing to the umami taste when combined with amino acids.[51] Among them, 5′-ribonucleotides—guanosine monophosphate (GMP), inosine monophosphate (IMP), and adenosine monophosphate (AMP)—exhibit strong synergistic effects with L-glutamate, markedly intensifying the umami sensation.[52] Differences observed in nucleotide concentrations suggest that the strains varied in their ability to generate or accumulate flavor-active nucleotides during the fermentation of prawn by-product juice (Table 2). Across the fermentation period of five days, all strains except *L. plantarum* led to a notable increase in the concentration of total umami-active 5′-nucleotides, including IMP, GMP and AMP (Fig. 4). Flavor nucleotides are generated through the metabolic activity of microorganisms, which degrade adenosine triphosphate (ATP) and nucleic acids *via* enzymes like nucleases (phosphodiesterase) into taste-active 5′-nucleotides.[53]

Among the species tested, *R. oligosporus* produced the highest total flavor nucleotide content by day 3, suggesting more rapid metabolic turnover during the early stages of fermentation (Fig. 4). *A. oryzae*, another filamentous fungus known to produce extracellular nucleases, was also among the higher nucleotide producers in this study, although its levels remained below those of *R. oligosporus* (Table 2).[54] This is consistent with previous studies showing that *Rhizopus* spp. could rapidly colonize substrates and secrete extracellular enzymes during tempeh fermentation.[55–57] In addition, *R. oligosporus* has been reported to produce extracellular ribonucleases, indicating a potential role for RNA degradation in the early accumulation of nucleotides.[58] Broader fungal evidence from *A. oryzae* further supports this, in which S1/P1-type nucleases hydrolyze single-stranded nucleic acids and release mononucleotide products.[54] Taken together, these findings suggest that RNA turnover may contribute to the early nucleotide accumulation observed in the fermentation driven by *R. oligosporus*, although the specific formation pathways of AMP, GMP, and IMP in *R. oligosporus* have yet to be conclusively

Table 1 Free amino acid contents (mg mL^{-1}) of the fermented prawn juice using different microorganisms[a,b,c,d]

Amino acids	AO					RO				SC			
	D0	D1	D2	D3	D5	D1	D2	D3	D5	D1	D2	D3	D5
Total	4.29 ± 0.15^{abcd}	3.98 ± 0.367^{abcdef}	2.99 ± 0.07^{cdef}	4.37 ± 0.27^{abcd}	4.01 ± 0.37^{abcdef}	4.09 ± 0.72^{abcdef}	3.08 ± 0.17^{bcdef}	4.49 ± 0.77^{ab}	4.60 ± 0.37^{a}	4.43 ± 0.46^{abc}	2.91 ± 0.19^{def}	4.21 ± 0.31^{abcde}	3.24 ± 0.15^{bcdef}
Umami	0.12 ± 0.01^{fg}	0.10 ± 0.003^{fg}	0.23 ± 0.03^{fg}	0.52 ± 0.09^{de}	0.72 ± 0.11^{ab}	0.11 ± 0.001^{fg}	0.24 ± 0.01^{f}	0.44 ± 0.08^{e}	0.76 ± 0.07^{a}	0.12 ± 0.005^{fg}	0.21 ± 0.01^{fg}	0.50 ± 0.03^{de}	0.47 ± 0.08^{de}
Sweet	1.08 ± 0.02^{abc}	1.04 ± 0.04^{abc}	0.94 ± 0.02^{abc}	1.27 ± 0.07^{ab}	0.76 ± 0.27^{bc}	1.08 ± 0.10^{abc}	0.94 ± 0.18^{abc}	1.23 ± 0.36^{ab}	0.92 ± 0.38^{abc}	1.18 ± 0.15^{abc}	0.91 ± 0.07^{abc}	1.20 ± 0.05^{ab}	0.66 ± 0.24^{c}
Bitter	1.49 ± 0.03^{abcde}	1.32 ± 0.22^{abcdefg}	1.09 ± 0.04^{efg}	1.40 ± 0.10^{abcdefg}	1.28 ± 0.19^{abcdefg}	1.18 ± 0.10^{defg}	1.15 ± 0.04^{defg}	1.48 ± 0.21^{abcde}	1.67 ± 0.42^{abc}	1.57 ± 0.17^{abcd}	1.10 ± 0.11^{defg}	1.35 ± 0.10^{abcdefg}	1.13 ± 0.09^{defg}

Amino acids	BS				LP				LS			
	D1	D2	D3	D5	D1	D2	D3	D5	D1	D2	D3	D5
Total	3.54 ± 0.27^{abcdef}	2.80 ± 0.25^{f}	4.21 ± 0.87^{abcde}	3.85 ± 0.62^{abcdef}	3.75 ± 0.28^{abcdef}	2.94 ± 0.15^{def}	4.84 ± 0.81^{a}	4.85 ± 0.36^{a}	3.09 ± 0.15^{cdef}	2.73 ± 0.16^{f}	3.84 ± 0.17^{abcdef}	3.99 ± 0.17^{abcdef}
Umami	0.09 ± 0.01^{g}	0.20 ± 0.03^{fg}	0.46 ± 0.08^{de}	0.55 ± 0.07^{cde}	0.10 ± 0.01^{fg}	0.21 ± 0.001^{fg}	0.58 ± 0.03^{bcd}	0.68 ± 0.05^{abc}	0.09 ± 0.003^{g}	0.21 ± 0.01^{fg}	0.51 ± 0.04^{de}	0.67 ± 0.01^{abc}
Sweet	0.93 ± 0.04^{abc}	0.80 ± 0.13^{abc}	1.08 ± 0.38^{abc}	0.76 ± 0.30^{bc}	0.98 ± 0.04^{abc}	0.91 ± 0.06^{abc}	1.29 ± 0.20^{a}	1.07 ± 0.06^{abc}	0.93 ± 0.03^{abc}	0.90 ± 0.06^{abc}	1.09 ± 0.07^{abc}	0.89 ± 0.06^{abc}
Bitter	1.30 ± 0.08^{abcdefg}	1.15 ± 0.13^{defg}	1.47 ± 0.37^{abcdef}	1.33 ± 0.22^{abcdefg}	1.34 ± 0.06^{abcdefg}	1.24 ± 0.05^{bcdefg}	1.70 ± 0.20^{ab}	1.75 ± 0.10^{a}	0.97 ± 0.02^{g}	1.00 ± 0.05^{fg}	1.21 ± 0.06^{cdefg}	1.28 ± 0.08^{abcdefg}

[a] $N = 3$ fermentation trials. [b] Mean values ± standard deviation with different lowercase superscript letters within the same row are significantly different (two-way ANOVA, Tukey test, and $p < 0.05$). [c] Umami = Glu + Asp; Sweet = Thr + Ser + Gly + Ala + Pro; Bitter = Val + Met + Ile + Leu + Phe + His + Arg. [d] AO – *A. oryzae*, RO – *R. oligosporus*, SC – *S. cerevisiae*, BS – *B. subtilis*, LP – *L. plantarum*, LS – *L. sakei*.

Table 2 Flavor nucleotides (mg L^{-1}) of the fermented prawn juice using different microorganisms[a,b,c,d]

	AO				RO				SC			
	D0	D1	D2	D3	D5	D1	D2	D3	D5	D1	D2	D3	D5
AMP	0.36 ± 0.002^{ab}	0.37 ± 0.02^{ab}	0.42 ± 0.04^{ab}	0.41 ± 0.04^{ab}	0.38 ± 0.04^{ab}	0.52 ± 0.09^{ab}	0.49 ± 0.10^{ab}	0.64 ± 0.26^{a}	0.55 ± 0.24^{ab}	0.47 ± 0.01^{ab}	0.43 ± 0.04^{ab}	0.40 ± 0.02^{ab}	0.28 ± 0.22^{b}
IMP	0.40 ± 0.02^{cd}	0.55 ± 0.04^{abcd}	0.52 ± 0.05^{abcd}	0.54 ± 0.06^{abcd}	0.80 ± 0.09^{a}	0.54 ± 0.02^{abcd}	0.53 ± 0.07^{abcd}	0.57 ± 0.09^{abcd}	0.76 ± 0.15^{ab}	0.62 ± 0.02^{abcd}	0.56 ± 0.03^{abcd}	0.61 ± 0.01^{abcd}	0.55 ± 0.45^{abcd}
GMP	0.12 ± 0.04^{bc}	0.15 ± 0.13^{bc}	0.04 ± 0.07^{c}	ND	0.04 ± 0.07^{c}	0.65 ± 0.05^{abc}	0.84 ± 0.34^{ab}	1.10 ± 0.94^{a}	0.57 ± 0.60^{abc}	0.09 ± 0.01^{c}	0.04 ± 0.06^{c}	0.01 ± 0.01^{c}	0.11 ± 0.20^{c}
Total	0.88 ± 0.03^{bc}	1.07 ± 0.08^{bc}	0.97 ± 0.04^{bc}	0.95 ± 0.04^{bc}	1.22 ± 0.09^{bc}	1.70 ± 0.14^{abc}	1.87 ± 0.39^{ab}	2.31 ± 1.16^{a}	1.87 ± 0.82^{ab}	1.18 ± 0.02^{bc}	1.04 ± 0.06^{bc}	1.02 ± 0.01^{bc}	0.95 ± 0.80^{bc}
	BS				LP				LS				
	D1	D2	D3	D5	D1	D2	D3	D5	D1	D2	D3	D5	
AMP	0.45 ± 0.04^{ab}	0.47 ± 0.05^{ab}	0.49 ± 0.13^{ab}	0.43 ± 0.01^{ab}	0.41 ± 0.03^{ab}	0.44 ± 0.03^{ab}	0.43 ± 0.05^{ab}	0.42 ± 0.08^{ab}	0.39 ± 0.02^{ab}	0.40 ± 0.01^{ab}	0.43 ± 0.03^{ab}	0.43 ± 0.03^{ab}	
IMP	0.55 ± 0.003^{abcd}	0.51 ± 0.08^{abcd}	0.53 ± 0.09^{abcd}	0.63 ± 0.04^{abc}	0.43 ± 0.07^{cd}	0.32 ± 0.03^{cd}	0.31 ± 0.01^{d}	0.34 ± 0.03^{cd}	0.45 ± 0.06^{bcd}	0.36 ± 0.06^{cd}	0.41 ± 0.04^{cd}	0.45 ± 0.03^{bcd}	
GMP	0.24 ± 0.08^{bc}	0.20 ± 0.13^{bc}	0.21 ± 0.18^{bc}	0.23 ± 0.15^{bc}	0.08 ± 0.05^{c}	0.08 ± 0.09^{c}	0.11 ± 0.11^{c}	0.12 ± 0.07^{bc}	0.09 ± 0.08^{c}	0.01 ± 0.02^{c}	0.02 ± 0.02^{c}	0.11 ± 0.08^{c}	
Total	1.23 ± 0.09^{bc}	1.19 ± 0.24^{bc}	1.24 ± 0.37^{bc}	1.30 ± 0.18^{abc}	0.91 ± 0.09^{bc}	0.85 ± 0.12^{bc}	0.85 ± 0.14^{bc}	0.87 ± 0.12^{bc}	0.93 ± 0.04^{bc}	0.77 ± 0.07^{c}	0.86 ± 0.02^{bc}	0.99 ± 0.04^{bc}	

[a] $N = 3$ fermentation trials. [b] Mean values ± standard deviation with different lowercase superscript letters within the same row are significantly different (two-way ANOVA, Tukey test, and $p < 0.05$). [c] ND (not detected and < limit of quantification). [d] AO – *A. oryzae*, RO – *R. oligosporus*, SC – *S. cerevisiae*, BS – *B. subtilis*, LP – *L. plantarum*, LS – *L. sakei*.

Fig. 4 Changes in total flavor nucleotide concentrations of the prawn by-product juice fermented with different microbial strains throughout the fermentation period. Lowercase superscript letters represent significantly different values (two-way ANOVA, Tukey test, $p < 0.05$).

established. The elevated levels of IMP and GMP concentration are particularly important, as these compounds interact strongly with umami amino acids, enhancing the overall taste intensity.[59]

However, the total flavor nucleotides in fermented prawn by-product juice by *S. cerevisiae* and *L. plantarum* remained essentially unchanged over the 5-day fermentation period, while those by *B. subtilis* and *L. sakei* showed only a small overall increase that is not significantly different from the sample at day 0 ($p > 0.05$). The limited accumulation of flavor nucleotides in these microbial fermentations may reflect a balance between nucleotide generation and further metabolism, rather than sustained accumulation. In *S. cerevisiae*, the accumulation of flavor-enhancing nucleotides, is regulated by the purine biosynthetic pathway.[60] Ribose-5-phosphate, derived from the pentose phosphate pathway *via* RKI1, serves as a key precursor for nucleotide formation and is subsequently converted into IMP.[61] It can be further metabolized toward GMP or AMP *via* different enzymatic pathways.[62] Similarly, the regulation of IMP, GMP and AMP in *B. subtilis* is regulated by the purine nucleotide metabolic pathway.[63,64] The purine and pyrimidine metabolic pathways not only support the overall synthesis of nucleotides but also enable the conversion between nucleobases, nucleosides, and nucleotides, helping maintain an appropriate balance among these end products.[65] In LAB, nucleotide biosynthesis, salvage, and interconversion pathways have been well established, suggesting that released nucleotide-derived compounds may have been rapidly interconverted or taken up by LAB.[66] However, the specific mechanisms governing nucleotide accumulation in the present study were not directly measured.

3.4. Umami potential: TAV and EUC analysis

To evaluate the relative contribution of individual compounds to taste perception, the taste activity values (TAVs) and

equivalent umami concentrations (EUCs) were calculated (Table 3). The TAVs for 5′-AMP, 5′-IMP and 5′-GMP are not shown as the values are less than 0.01 for all samples. Among the major umami-related compounds, only glutamic acid contributed perceptibly to the overall taste at a concentration above its sensory threshold after fermentation (TAV >1).

According to Mau,[67] the EUC values can be classified into 4 levels: first level (>10 g MSG per g dry matter), second level (1–10 g MSG per g), third level (0.1–1 g MSG per g), and fourth level (0.1 g MSG per g). The EUC values increased markedly from 0.23 g MSG/100 g at day 0 to 6.36 g MSG/100 g on day 3 in the samples fermented with *R. oligosporus*. Among the tested microorganisms, fungal fermentations generated higher EUC values; *R. oligosporus* fermentation yielded the highest EUC value, corresponding to the second-level range, followed by *A. oryzae*. This corresponds with increases in glutamic acid TAVs, indicating strong synergistic effects between free amino acids and flavor nucleotides in these samples. Notably, the EUC value of the *R. oligosporus* fermented prawn juice on day 3 was comparable to that reported for fresh whiteleg shrimp meat (6.56 g MSG/100 g),[51] but higher than those of Chinese mitten crab (4.2 g MSG/100 g),[59] boiled whiteleg shrimp meat (4.11 g MSG/100 g)[51] and Chinese shrimp (4.58 g MSG/100 g).[68] This suggests that short-term fungal fermentation of prawn heads, a waste stream, can generate 'meat-like' levels of umami intensity on par with, or even greater than, those found in fresh seafood.

Phat *et al.*[69] reported a significant positive correlation between EUC values, electronic tongue sensory scores, and human sensory evaluations, supporting the use of EUC as a reliable indicator of umami intensity. The elevated EUC values observed in this study, therefore, demonstrate that microbial fermentation effectively enhances the umami characteristics of prawn by-products, transforming them into flavor-rich substrates.

Table 3 EUCs (g MSG/100 g) and TAVs of the umami-related compounds in the fermented prawn juice using different microorganisms[a,b,c]

			TAV	
		EUC (g MSG/100 g)	Glu	Asp
	D0	0.23 ± 0.04^{b}	0.12 ± 0.01^{c}	0.08 ± 0.01^{h}
AO	D1	0.27 ± 0.03^{b}	0.11 ± 0.02^{c}	0.072 ± 0.003^{h}
	D2	0.66 ± 0.08^{b}	0.39 ± 0.06^{c}	0.11 ± 0.01^{gh}
	D3	1.50 ± 0.31^{b}	0.94 ± 0.17^{b}	0.24 ± 0.04^{bcde}
	D5	2.86 ± 0.69^{b}	1.33 ± 0.25^{ab}	0.32 ± 0.04^{ab}
RO	D1	0.45 ± 0.06^{b}	0.10 ± 0.02^{c}	0.079 ± 0.004^{h}
	D2	1.88 ± 0.50^{b}	0.40 ± 0.04^{c}	0.12 ± 0.01^{fgh}
	D3	6.36 ± 3.71^{a}	1.24 ± 0.23^{ab}	0.07 ± 0.02^{h}
	D5	6.00 ± 2.31^{a}	1.64 ± 0.52^{a}	0.27 ± 0.11^{abcd}
SC	D1	0.26 ± 0.01^{b}	0.11 ± 0.01^{c}	0.09 ± 0.01^{h}
	D2	0.62 ± 0.09^{b}	0.34 ± 0.02^{c}	0.10 ± 0.01^{gh}
	D3	1.59 ± 0.38^{b}	0.93 ± 0.24^{b}	0.18 ± 0.02^{efg}
	D5	2.27 ± 1.74^{b}	1.14 ± 0.12^{b}	0.13 ± 0.05^{fgh}
BS	D1	0.25 ± 0.02^{b}	0.090 ± 0.001^{c}	0.07 ± 0.01^{h}
	D2	0.82 ± 0.35^{b}	0.35 ± 0.08^{c}	0.09 ± 0.01^{h}
	D3	2.48 ± 0.82^{b}	1.05 ± 0.03^{b}	0.26 ± 0.03^{abcd}
	D5	2.80 ± 0.83^{b}	1.09 ± 0.16^{b}	0.22 ± 0.02^{cde}
LP	D1	0.19 ± 0.03^{b}	0.097 ± 0.004^{c}	0.071 ± 0.005^{h}
	D2	0.52 ± 0.11^{b}	0.33 ± 0.01^{c}	0.109 ± 0.005^{gh}
	D3	1.55 ± 0.53^{b}	0.95 ± 0.17^{b}	0.22 ± 0.01^{cde}
	D5	1.95 ± 0.11^{b}	1.15 ± 0.11^{b}	0.34 ± 0.02^{a}
LS	D1	0.19 ± 0.02^{b}	0.097 ± 0.004^{c}	0.064 ± 0.003^{h}
	D2	0.43 ± 0.03^{b}	0.31 ± 0.02^{c}	0.112 ± 0.006^{gh}
	D3	1.46 ± 0.07^{b}	1.02 ± 0.07^{b}	0.20 ± 0.01^{def}
	D5	2.25 ± 0.27^{b}	1.23 ± 0.04^{ab}	0.30 ± 0.02^{abc}

[a] $N = 3$ fermentation trials. [b] Mean values ± standard deviation with different lowercase superscript letters within the same column are significantly different (two-way ANOVA, Tukey test, and $p < 0.05$). [c] AO – *A. oryzae*, RO – *R. oligosporus*, SC – *S. cerevisiae*, BS – *B. subtilis*, LP – *L. plantarum*, LS – *L. sakei*.

4. Conclusion

This study demonstrates that microbial fermentation is an effective strategy to enhance the umami characteristics of prawn by-products, contributing to their valorization as sustainable flavoring ingredients. Among the tested microorganisms, *R. oligosporus* showed the highest efficiency in accumulating umami-related compounds, including glutamic acid, aspartic acid, and flavor nucleotides, resulting in the highest EUC. These findings provide a scientific basis for converting prawn processing residues into high-value, flavor-enhancing ingredients through microbial biotransformation. This can potentially contribute to the circular economy, by harnessing microbial potential for net-zero waste. Nevertheless, this study was conducted under controlled laboratory conditions without sensory validation, which may limit direct translation to industrial applications. In addition, the interactions among flavor-active compounds were inferred from compositional data rather than confirmed through integrated sensory or metabolic analyses. Future work may focus on process optimization, scale-up feasibility, and comprehensive sensory evaluation to support the development of sustainable, prawn-based flavor enhancers for commercial applications.

Author contributions

Cia Min Lim: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft. Ying Tong Yeo: methodology, validation, writing—review & editing. Cherie Chin: methodology, validation, writing—review & editing. Wei Ning Chen: conceptualization, funding acquisition, project administration, supervision, validation, writing—review & editing.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Data availability

The manuscript and supplementary information (SI) contain all data necessary to support the findings, statements, and conclusions. Data will be made available on request.

Supplementary information is available. See DOI: https://doi.org/10.1039/d5fb00939a.

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