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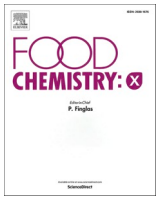

Effect of *Lactobacillus plantarum* and flavourzyme on protein degradation and flavor development in grass carp during fermentation

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ABSTRACT

This study examined the effect of Flavourzyme and *Lactobacillus plantarum* (*L. plantarum*) on protein degradation and flavor development during grass carp fermentation. The control groups comprised natural fermentation and fermentation with L. *plantarum*. Compared with the two control samples, those exposed to combined Flavourzyme and L. *plantarum* fermentation exhibited lower moisture content and enhanced protein hydrolysis, which accelerated the production of water-soluble taste substances (trichloroacetic acid-soluble peptides and free amino acids). The electronic tongue and electronic nose results indicated that the grass carp subjected to combined fermentation way displayed a more intense umami taste and aroma. Moreover, the sensory evaluation results confirmed that the combined fermentation method significantly improved the taste and odor attributes of fermented grass carp. In conclusion, combined fermentation with Flavourzyme and L. *plantarum* may effectively reduce fermentation time and enhance the flavor of fermented grass carp products.

1. Introduction

Grass carp (*Ctenopharyngodon idellus*), the largest freshwater fish species in China, are widely farmed throughout Southeast Asia due to their rapid growth rate and high nutritional value (Yang et al., 2020; Yang et al., 2020). The Chinese aquaculture production of grass carp has experienced significant recent growth, increasing from 4.222 million tons in 2010 to 5.90 million tons in 2022 (FAO, 2021). However, the perishable nature of grass carp restricts processing, meaning that most of the fish are sold at lower prices as live bodies or primary processed products. Limiting the processing utilization rate hinders the development of the industry.

Fermented foods are known for their distinctive flavors, nutritional richness, and long shelf life, making them highly popular among consumers (Marco et al., 2021). In recent years, fermented fish products have attracted significant attention, prompting extensive research on traditional fish paste products, fish fillets, and whole fish using biotechnological fermentation techniques. The common fermentation approaches currently used include natural fermentation (Zhao et al., 2021), individual or multiple bacterial inoculation (Hua, Sun, Xu, Gao, & Xia, 2022; Lv et al., 2023; Zhang, Hu, Xie, & Wang, 2020), and Flavourzyme addition (Yang, Jiang, et al., 2020; Yang, Liu, et al., 2020). Fermentation via inoculation is frequently used during fish processing since the ability to control the selection of an appropriate initiator can facilitate better flavor formation (Zhao et al., 2021). Lactic acid bacteria, Staphylococcus, and Saccharomyces, which are confirmed as beneficial starters, play a crucial role in enhancing the overall quality of related fish products (Yang, Jiang, et al., 2020; Yang, Liu, et al., 2020; Zeng, Chen, & Zhang, 2016; Zhao et al., 2021). However, studies have shown that lactic acid bacteria display relatively low enzyme activity, which can negatively affect the maturation cycle of fermented products (Yang, Xia, Zhang, Xu, & Jiang, 2016). Therefore, research currently focuses on effectively reducing the maturation period of fermented products during inoculation fermentation using lactic acid bacteria.

Adding Flavourzyme during fermentation represents a potential approach for reducing fermentation time and improving the nutritional

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value and flavor quality of fermented products (Feng et al., 2014; Xiao, Xu, Guo, & Shi, 2022; Zhang et al., 2017). However, limited research is available on the application of Flavourzyme during fish processing via fermentation, particularly fermented grass carp products. Our previous study found that *Lactiplantibacillus* represented the dominant strain in fermented grass carp (Xiao et al., 2023), while combining Flavourzyme and *Lactobacillus plantarum* (*L. plantarum*) during grass carp fermentation significantly enhanced its safety and physicochemical quality (Xu, Xiao, Xu, Guo, & Shi, 2022). Flavor crucially affects consumer perception (Afzal et al., 2022). However, the exact impact of Flavorzyme and L. *plantarum* on flavor development during grass carp fermentation remains unclear. Consequently, investigating the impact of Flavorzyme and *L. plantarum* in the flavor development of fermented grass carp is essential and may improve the industrial production of high-quality related products.

The relative protein degradation and sensory assessment indicators such as the moisture content, total nitrogen (TN), non-protein nitrogen (NPN), protein degradation index (PI), trichloroacetic acid (TCA)-soluble peptides, free amino acids (FAAs), electronic tongue (E-tongue), electronic nose (E-nose), and sensory properties were analyzed to investigate the effect of Flavourzyme and L. *plantarum* on the flavor formation in grass carp during fermentation. The control groups consist of samples exposed to natural fermentation (NF) and inoculation fermentation with L. *plantarum* (LF). This study may offer valuable insights for enhancing the fermentation techniques and producing high-quality fermented grass carp products.

2. Materials and methods

2.1. Materials

Twenty fresh grass carp (*Cyprinidae*) weighing 2.5–3.0 kg each were acquired from a local aquatic products market near Shanghai Ocean University (Shanghai, China). The dorsal meat of the fish was collected and cut into 3 cm (length) × 2 cm (width) × 2 cm (height) cubes for subsequent fermentation. The L. *plantarum* (No. 192567) was purchased from Beijing Beina Tronlink Biotechnology Research Institute (Beijing, China), while the Flavourzyme, with enzyme activity of 30,000 U/g, was obtained from Beijing Solebo Technology Co. Ltd. (Beijing, China). Food-grade corn was acquired from Zhejiang Yiwan Biotechnology Co. Ltd. All other chemicals were analytically pure and supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation of samples

The fermented samples were prepared using a previously described method (H. Xu et al., 2022). The grass carp meat was cured for 24 h at 4 °C with a mixture of salt (3%, *w*/w) and sugar (2%, w/w), after which it was thoroughly mixed with roasted corn flour (20%, w/w). Next, the meat was divided into three batches, and different starter cultures were added, which included NF, LF (1% *L. plantarum*), and FLF (a combination of 10 U/g Flavourzyme and 1% *L. plantarum*). The NF and LF samples served as the control. The fish samples, corn, and starter cultures were combined in a ceramic vessel and sealed via immersion in water. The samples were placed in an incubation chamber at 25 °C and a humidity level of 75% for cycles of 0 d, 5 d, 10 d, and 15 d, respectively. Finally, the cornmeal was removed, and the fermented samples were stored at −80 °C for subsequent experimental analysis.

2.3. Moisture content analysis

The moisture content was determined using the standard method 930.15 of the AOAC (2005).

2.4. TN analysis

The TN was determined using AOAC 984.13 (AOAC, 2005). Here, 0.2 g of the samples were weighed into 2 mL sulfuric acid (98%, *w*/w), potassium sulfate (3.50 g), and copper sulfate (0.40 g), and digested using an automated Kjeltec 8400 Kjeldahl analyzer (FOSS, Hillerød, Denmark). After digestion, the samples were transferred and dissolved in boric acid (1%, *w/v*), followed by titration with hydrochloric acid (0.1 mol L^{-1}).

2.5. NPN and PI analysis

The NPN was determined using a method described by Wang et al. (2021) with slight modifications. 2 g of the fermented sample was homogenized with 18 mL of distilled water for 1 min and centrifuged at 2000 ×*g* for 10 min, after which the supernatant was filtered. The accumulated filtrate (10 mL) was combined with a 10 mL TCA dilution (20%, w/v) and filtered after a 30 min residence time. The PI was calculated using Formula (1):

$$\mathrm{pI} = \frac{\mathrm{TN}}{\mathrm{NPN}} \times 100\% \quad (1)$$

2.6. TCA-soluble peptide analysis

The TCA-soluble peptides were analyzed using a previously delineated method (Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004), while the content was determined using a bioreagent technique. The results were presented as molar equivalents of Tyrosine (Tyr) per gram of sample (μmol Tyr/g).

2.7. FAA analysis

The FAA content was determined using a method described by Yu et al. (2018). 2 g sample was weighed into 10% TCA(*w/v*), homogenized for 1 min, and centrifuged, after which the pH of the supernatant was fixed at 2.0. The sample solution was dissolved in distilled water to 10 mL and filtered through 0.22 μm membranes for further FAA analysis.

2.8. E-tongue analysis

The taste profiles of samples were assessed using an E-tongue (Isenso, Super Tongue, France) equipped with eighteen electrochemical probes sensitive to five taste attributes of a test sample, including bitterness, freshness, saltiness, sourness, and sweetness. The test was performed according to a procedure outlined by Qing et al. (2020). The 2 g sample was thoroughly mixed with 15 mL of ultrapure water and filtered through a neutral filter paper. The filtrate was diluted to a final volume of 100 mL with ultrapure water for subsequent E-tongue analysis.

2.9. E-nose analysis

The odor profiles of the samples were assessed using a Fox 4000 Sensory electronic nose analysis system (Alpha M.O.S., Toulouse, France) equipped with 18 metal oxide sensors, which were divided into three types (L-, P-, and T-type) based on their different characteristics (Gu et al., 2019). A comprehensive overview of the sensors is provided in Supplemental Table S1. The E-nose analysis was performed using a method introduced by Wang, Zhang, Zhu, Wang, and Shi (2018).

2.10. Sensory assessment

The sensory experiment in this study was approved by the Ethics Committee of Shanghai Ocean University (Approval Number: SHOU-DW-2023-090). A panel of 15 expert evaluators with experience in the sensory evaluation of fermented fish products assessed the sensory

quality of the samples using quantitative descriptive analysis (QDA). The specific criteria for the sensory evaluation are provided in Supplemental Table S2. Five sample attributes were evaluated including color, odor, taste, texture, and overall acceptability (Kiran et al., 2023). The intensity of these attributes was rated on a sequential 10-point scale.

2.11. Data analysis

All tests were performed in triplicate. The results were presented as the mean ± standard deviation (SD). The statistical analysis was performed using the SPSS 21.0 software (IBM, Armonk, NY, USA) with one-way analysis of variance (ANOVA), and Duncan's multiple range test was employed to determine significance ($p < 0.05$). The diagrams were plotted using the Origin 2019b software (Origin Lab Corporation, Northampton, NY, USA).

3. Results and discussion

3.1. Moisture content analysis

Fig. 1 shows the variation in the moisture content of the fermented samples in the NF, LF, and FLF groups. The moisture levels in all the groups decreased as fermentation progressed (Fig. 1A), which was possibly linked to a lower water-holding capacity in the grass carp samples after fermentation. Studies have shown that microbial proliferation can significantly reduce the pH and increase protein degradation during fish fermentation, decreasing the water retention capacity of fish proteins (Huff-Lonergan & Lonergan, 2005; Jittrepotch, Rojsuntornkitti, & Kongbangkerd, 2015). Furthermore, the samples in the FLF group displayed a significantly lower moisture content during each fermentation period compared to the NF and LF samples, indicating that combined Flavourzyme and L. *plantarum* fermentation exacerbated the moisture loss from fermented grass carp.

3.2. Protein degradation analysis

The TN, NPN, and PI levels can accurately represent the protein degradation of fish meat during the fermentation process (Wang et al., 2021; Xu et al., 2018). Figs. 1B-D illustrate the variation between the TN and NPN content and PI values of the samples in NF, LF, and FLF groups during fermentation. No significant differences were evident between the TN levels of the samples, indicating that fermentation did not affect the TN content. However, the NPN content and PI values increased significantly in all group samples as fermentation progressed, suggesting protein degradation and peptide generation in fermented grass carp samples. After 15 d of fermentation, the PI values of samples in the NF, LF, and FLF groups increased from 5.28 ± 0.36% to 7.50 ± 0.26, 8.77 ± 0.18% and 11.40 ± 0.27%, respectively. This suggested that combined Flavorzyme and L. *plantarum* fermentation accelerated protein hydrolysis in the grass carp samples, as evidenced by a more substantial increase in the PI values of the FLF group (nearly 1.50-fold of the PI values in the FLF group than that of the NF group).

3.3. TCA-soluble peptides analysis

Measuring the TCA-soluble peptides levels is vital for evaluating the abundance of small molecular peptides and the degree of protein hydrolysis (Sun et al., 2020; Yang, Jiang, et al., 2020; Yang, Liu, et al., 2020). The changes in the TCA soluble peptide content of the three groups were analyzed during the fermentation (Fig. 1E). The TCA soluble peptide levels in the NF, LF, and FLF groups increased as fermentation progressed. A similar trend was reported by Sriket (2014), who showed that the involvement of endogenous enzymes and microbial proteases was associated with an increase in the TCA soluble peptides. It is noteworthy that the FLF group displayed significantly higher TCA soluble peptide levels (10.78 ± 0.27 Tyr/g) at the end of fermentation (15 d), followed by the FL (8.56 ± 0.35 μmol Tyr/g) and NF (9.16 ± 0.10 Tyr/g) group. This suggested that combining Flavourzyme and L. *plantarum* could facilitate protein hydrolysis and the small-molecule

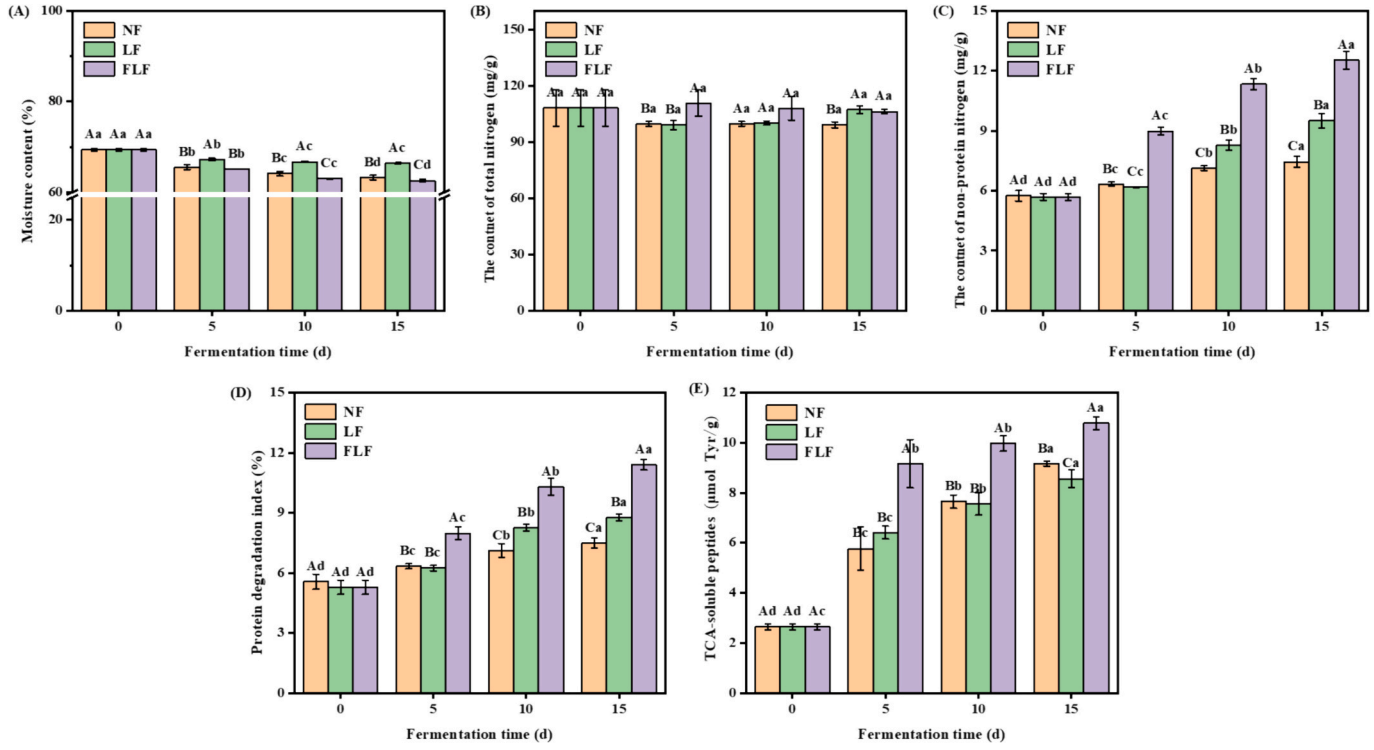

Fig. 1. Changes in moisture content (A), total nitrogen (B), non-protein nitrogen (C), protein degradation index(D), and TCA-soluble peptides (E) in fermented grass carp from NF, LF, and FLF groups during fermentation.

peptide formation in the fermented grass carp during fermentation.

3.4. FAA analysis

FAAs are crucial components derived from protein hydrolysis, contributing significantly to the nutritional value and taste quality of fish-based products (Jing et al., 2023; Ye et al., 2024). The concentration, composition, and threshold of FAAs are closely related to the taste of fish products (Xiao et al., 2021). Table 1 presents the FAAs detected in the NF, LF, and FLF groups during the fermentation. The results showed that 17 FAAs were identified in the three fermentation groups, including sweet amino acids (tyrosine (Thr), serine (Ser), glycine (Gly), alanine (Ala), and proline (Pro)), umami amino acids (aspartic acid (Asp) and glutamic acid (Glu)), and bitter amino acids (cysteine (Cys), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), and arginine (Arg)) (Wu et al., 2020; Xiang, Xia, Fang, & Zhong, 2024; Xiao et al., 2021). Extending the fermentation time, the FAA levels in the NF, LF, and FLF groups significantly increased, whereas the Pro content displayed an initial decline, followed by an increase. Furthermore, the His content decreased substantially in the three groups at the end of fermentation, possibly due to the free His decarboxylation process during the fermentation (Gagic et al., 2019). Additionally, to comprehensively examine the effect of L. *plantarum* and Flavourzymes on the FAA content during grass carp fermentation, the total umami and sweet amino acid (TUSAA), total bitter amino acid (TBAA), and total amino acid (TAA) content in the NF, LF, and FLF groups were determined at all the fermentation stages (Fig. 2). Notably, Although the TUSAA, TBAA, and TAA levels in the three groups samples increased gradually as fermentation progressed, they were significantly higher in the FLF group at

Table 1
The changes in free amino acids composition and concentration (db, mg/100 g) in fermented grass carp samples from NF, LF, and FLF groups during fermentation.

Types	Taste characteristics	Threshold (mg/100 g)	Samples	Fermentation time (d)			
				0	5	10	15
			NF	13.69 ± 0.32^{Ad}	62.65 ± 3.71^{Bc}	106.70 ± 2.12^{Bb}	199.75 ± 10.69^{Ba}
Asparagine	umami (+)	100	LF	13.69 ± 0.32^{Ad}	52.35 ± 1.24^{Cc}	98.60 ± 2.74^{Cb}	144.45 ± 4.72^{Ca}
			FLF	13.69 ± 0.32^{Ad}	101.53 ± 9.68^{Ac}	194.01 ± 2.23^{Ab}	230.92 ± 19.40^{Aa}
			NF	52.81 ± 0.73^{Ad}	58.27 ± 1.80^{Bc}	70.04 ± 0.92^{Bb}	115.20 ± 4.26^{Ba}
Threonine	sweet(+)	260	LF	52.81 ± 0.73^{Ac}	38.22 ± 0.60^{Bd}	68.41 ± 2.54^{Bb}	107.42 ± 2.15^{Ba}
			FLF	52.81 ± 0.73^{Ad}	68.47 ± 3.50^{Ac}	128.96 ± 1.77^{Ab}	165.13 ± 13.13^{Aa}
			NF	8.98 ± 0.25^{Ab}	14.52 ± 2.24^{Aa}	4.15 ± 0.74^{Cc}	4.53 ± 0.47^{Cc}
Serine	sweet(+)	150	LF	8.98 ± 0.25^{Ab}	8.51 ± 0.53^{Bc}	9.18 ± 0.35^{Bb}	16.48 ± 1.28^{Aa}
			FLF	8.98 ± 0.25^{Ac}	12.09 ± 0.58^{Abc}	14.32 ± 0.67^{Ab}	20.57 ± 3.90^{Aa}
			NF	14.45 ± 0.26^{Ad}	161.42 ± 4.54^{Bc}	220.26 ± 3.31^{Bb}	319.52 ± 12.06^{Ba}
Glutamic	umami(+)	30	LF	14.45 ± 0.26^{Ad}	124.59 ± 3.26^{Cc}	192.51 ± 8.10^{Cb}	276.84 ± 4.84^{Ca}
			FLF	14.45 ± 0.26^{Ad}	199.43 ± 14.37^{Ac}	346.06 ± 2.20^{Ab}	410.48 ± 38.34^{Aa}
			NF	84.69 ± 2.83^{Ad}	88.01 ± 2.19^{Cc}	105.15 ± 3.70^{Cb}	143.66 ± 0.80^{Ca}
Glycine	sweet/umami(+)	130	LF	84.69 ± 2.83^{Ad}	131.28 ± 3.49^{Bc}	159.66 ± 6.74^{Bb}	198.67 ± 2.61^{Ba}
			FLF	84.69 ± 2.83^{Ad}	161.60 ± 4.58^{Ac}	203.53 ± 0.52^{Ab}	240.43 ± 17.08^{Aa}
			NF	39.91 ± 1.49^{Ad}	98.25 ± 1.80^{Bc}	152.28 ± 2.73^{ABb}	248.58 ± 2.39^{Ba}
Alanine	sweet/umami(+)	60	LF	39.91 ± 1.49^{Ad}	104.43 ± 23.19^{Ac}	147.18 ± 2.71^{Bb}	227.73 ± 6.04^{Ca}
			FLF	39.91 ± 1.49^{Ad}	131.40 ± 11.90^{Ac}	239.58 ± 2.75^{Ab}	305.59 ± 18.00^{Aa}
			NF	2.11 ± 0.40^{Ad}	12.44 ± 2.17^{Bc}	16.56 ± 1.68^{Bb}	9.23 ± 0.32^{Ca}
Cysteine	bitter/sweet/surfur(−)	ND	LF	2.11 ± 0.40^{Ad}	8.53 ± 1.17^{Cc}	12.30 ± 1.20^{Ca}	10.33 ± 0.54^{Bb}
			FLF	2.11 ± 0.40^{Ad}	17.21 ± 4.11^{Ac}	21.42 ± 2.11^{Ab}	29.11 ± 3.14^{Aa}
			NF	13.65 ± 0.19^{Ad}	61.19 ± 3.61^{Bc}	93.98 ± 1.21^{Bb}	143.12 ± 6.58^{Ba}
Valine	sweet/bitter(−)	40	LF	13.65 ± 0.19^{Ad}	64.14 ± 0.75^{Bc}	86.25 ± 4.64^{Cb}	129.20 ± 0.65^{Ca}
			FLF	13.65 ± 0.19^{Ad}	95.49 ± 6.22^{Ac}	172.39 ± 22.24^{Ab}	206.29 ± 12.49^{Aa}
			NF	4.83 ± 0.16^{Ad}	46.35 ± 2.60^{Cc}	78.85 ± 0.38^{Cb}	128.40 ± 5.86^{Ca}
Methionine	bitter/sweet/surfur(−)	30	LF	4.83 ± 0.16^{Ad}	63.57 ± 0.16^{Bc}	89.82 ± 5.42^{Bb}	136.06 ± 1.02^{Ba}
			FLF	4.83 ± 0.16^{Ad}	84.87 ± 5.11^{Ac}	152.13 ± 6.39^{Ab}	181.91 ± 22.44^{Aa}
			NF	10.72 ± 0.17^{Ad}	47.32 ± 3.05^{Cc}	78.51 ± 1.17^{Bb}	127.22 ± 7.60^{Ba}
Isoleucine	bitter(−)	90	LF	10.72 ± 0.17^{Ad}	58.26 ± 0.17^{Bc}	73.07 ± 3.35^{Cb}	106.94 ± 0.34^{Ca}
			FLF	10.72 ± 0.17^{Ac}	102.25 ± 33.54^{Ab}	140.41 ± 6.92^{Aa}	164.58 ± 21.52^{Aa}
			NF	22.44 ± 0.22^{Ad}	99.79 ± 2.85^{Cc}	176.14 ± 2.67^{Cb}	295.99 ± 13.94^{Ba}
Leucine	bitter(−)	190	LF	22.44 ± 0.22^{Ad}	132.71 ± 0.17^{Bc}	194.22 ± 5.58^{Bb}	294.11 ± 4.24^{Ba}
			FLF	22.44 ± 0.22^{Ad}	211.52 ± 35.80^{Ac}	327.77 ± 1.84^{Ab}	410.23 ± 39.79^{Aa}
			NF	23.66 ± 0.43^{Ad}	64.38 ± 1.84^{Cb}	61.71 ± 2.65^{Cb}	84.24 ± 0.75^{Ca}
Tyrosine	bitter(−)	ND	LF	23.66 ± 0.43^{Ad}	72.94 ± 0.02^{Bc}	98.06 ± 1.86^{Bb}	141.93 ± 2.48^{Ba}
			FLF	23.66 ± 0.43^{Ad}	109.54 ± 9.56^{Ac}	175.17 ± 13.16^{Ab}	201.27 ± 16.35^{Aa}
			NF	0.55 ± 0.14^{Ad}	99.37 ± 0.54^{Cc}	171.88 ± 1.08^{Cb}	315.02 ± 19.27^{Ca}
Phenylalanine	bitter(−)	90	LF	0.55 ± 0.14^{Ad}	158.09 ± 1.19^{Bc}	254.99 ± 0.53^{Bb}	404.64 ± 12.06^{Ba}
			FLF	0.55 ± 0.14^{Ad}	231.66 ± 20.64^{Ac}	398.26 ± 6.82^{Ab}	511.18 ± 39.24^{Aa}
			NF	57.71 ± 2.96^{Ad}	96.94 ± 0.82^{Cc}	125.91 ± 1.74^{Cb}	260.55 ± 23.33^{Ca}
Lysine	sweet/bitter(−)	50	LF	57.71 ± 2.96^{Ad}	149.73 ± 1.53^{Bc}	207.69 ± 2.04^{Bb}	296.99 ± 13.46^{Ba}
			FLF	57.71 ± 2.96^{Ad}	217.91 ± 20.85^{Ac}	334.67 ± 4.65^{Ab}	404.70 ± 21.63^{Aa}
			NF	762.54 ± 8.87^{Aa}	596.94 ± 13.18^{Bb}	516.42 ± 6.77^{Bc}	583.81 ± 32.66^{Ab}
Histidine	bitter(−)	20	LF	762.54 ± 8.87^{Aa}	663.86 ± 15.06^{Ab}	536.23 ± 21.95^{Bd}	585.88 ± 8.41^{Ac}
			FLF	762.54 ± 8.87^{Aa}	642.88 ± 35.21^{Ab}	630.00 ± 2.10^{Ab}	611.58 ± 43.48^{Ab}
			NF	100.57 ± 4.47^{Ab}	8.66 ± 0.46^{Cc}	9.50 ± 0.91^{Cc}	22.32 ± 1.09^{Cb}
Arginine	sweet/bitter(−)	50	LF	100.57 ± 4.47^{Ab}	75.30 ± 0.57^{Bd}	88.03 ± 1.31^{Bc}	124.74 ± 3.95^{Ba}
			FLF	100.57 ± 4.47^{Ac}	116.90 ± 8.17^{Ac}	167.83 ± 3.62^{Ab}	200.28 ± 19.64^{Aa}
			NF	82.83 ± 4.29^{Aa}	95.53 ± 1.51^{Ab}	123.68 ± 9.60^{Aa}	120.35 ± 4.34^{Aa}
Proline	sweet/bitter(+)	300	LF	82.83 ± 4.29^{Aa}	72.39 ± 1.29^{Bb}	70.37 ± 0.84^{Bb}	85.33 ± 3.54^{Ba}
			FLF	82.83 ± 4.29^{Ab}	92.35 ± 8.23^{Ab}	113.49 ± 5.77^{Aa}	117.47 ± 4.22^{Aa}

Note: (+) indicates pleasant taste; (−) denotes unpleasant taste; db indicates dry basis; ND represents that the threshold was not detected. The values that are presented reflect the average ± standard deviation. Variations in one column's letters indicate substantial differences ($P < 0.05$).

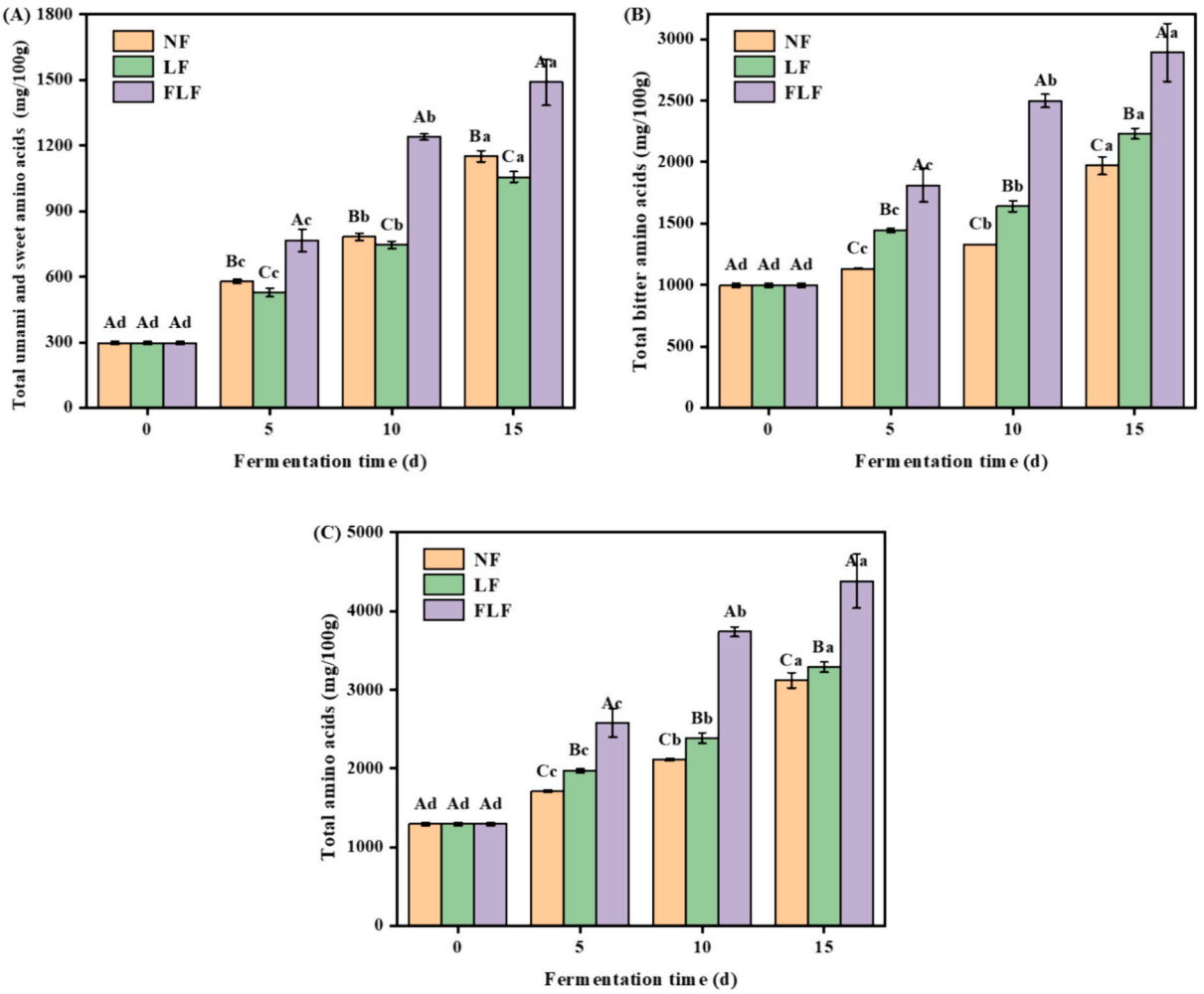

Fig. 2. Changes in content of total umami and sweet amino acids (A), total bitter amino acids (B), and total amino acids (C) in fermented grass carp from NF, LF, and FLF groups during fermentation.

each fermentation stage, followed by the FL and NF group. These results demonstrated that combined Flavourzyme and L. *plantarum* fermentation considerably enhanced taste attribute development in the fermented grass carp, improving the flavor profile.

3.5. E-tongue analysis

The E-tongue system is widely used for discriminating and analyzing the taste of samples (Qiu, Wang, & Gao, 2014). Principal component analysis (PCA) is a projection method that allows for the visualization of sample information and is specifically used to identify sample differences (Xiao et al., 2022). Fig. 3A illustrates the PCA results of the three groups during each fermentation period based on the response of the E-tongue electrodes. The results are presented in a two-dimensional scatterplot, with the two axes (PC1 and PC2) representing the overall taste profile of each fermented sample. The cumulative variance contribution rates of PC1 (80.11%) and PC2 (16.29%) reached 96.40%, indicating that these two elements corresponded to most of the taste profiles. Notably, the three fermentation groups displayed notable variations in taste characteristics, as evidenced by the distinct taste response distributions. Fig. 3B displays the taste radar map of the three groups during fermentation. The results showed that both the umami and sourness increased as fermentation progressed, possibly due to the accumulation of acids, particularly lactic acid. Related reports confirmed that lactic acid bacteria multiplied in fermented fish throughout the fermentation process, transforming carbohydrates into organic acids and reducing the pH of the fermentation product (Lin et al., 2021; Visessanguan et al., 2004). Meanwhile, the enhanced umami intensity may be associated with increased FAAs and TCA-soluble peptides, which are generated when proteins break down during fermentation (Fig. 1E and Fig. 2A). Furthermore, the FLF group exhibited a significantly higher umami intensity compared to the other two groups, indicating that L. *plantarum* and Flavorzyme co-inoculation during grass carp fermentation might enhanced umami flavor.

3.6. E-nose analysis

The E-nose system, a widely used method for detecting odor characteristics, is efficient for providing organoleptic information regarding food products (Wilson & Baietto, 2009). Fig. 3C shows the odor profiles of the three groups during each fermentation period, along with their PCA results. The PC1 (84.13%) and PC2 (10.39%) presented a cumulative variance contribution rate of 94.52%, indicating that each fermented sample displayed highly distinctive odor characteristics. Significant differences were evident between the aroma characteristics of the NF, LF and FLF groups during each fermentation period. Fig. 3D displays the odor radar patterns detected by the 18 odor sensors for samples from the NF, LF, and FLF groups. The volatile compounds of each fermented sample elicited stronger responses from P30/2 and PA/2, suggesting that the fermented grass carp might contain more alcohols, combustion products, aldehydes, hydrogen sulfide, amines, and ammonia. This result indicated that fermentation significantly enhanced

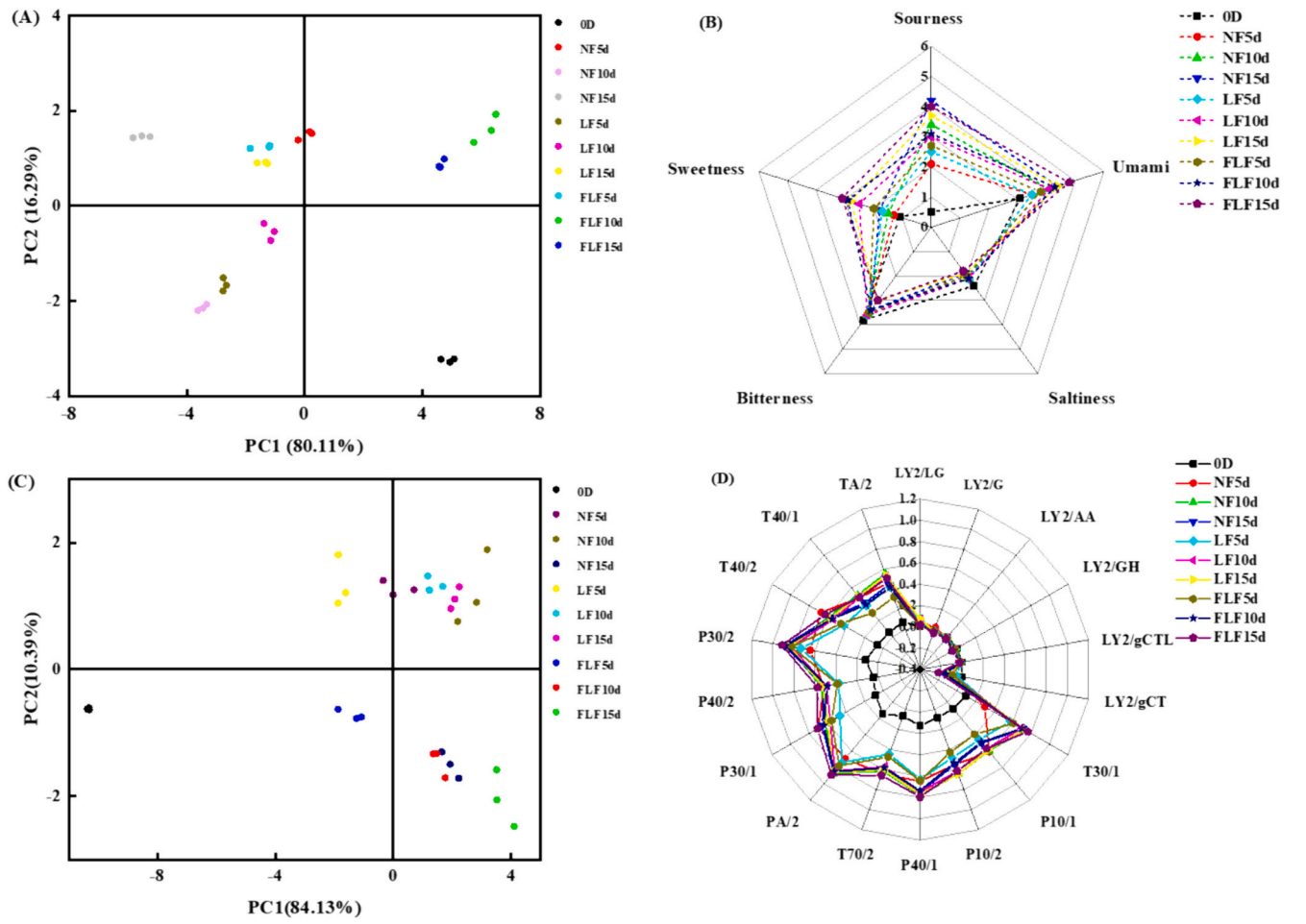

Fig. 3. *E*-tongue and E-nose analysis of fermented grass carp from NF, LF, and FLF groups during fermentation. (A) and (B) represent the PCA plot and radar graph of the E-tongue; (C) and (D) represent the PCA plot and radar graph of the E-nose. 0D indicates samples fermented for 0 days. NF 5d, NF 10d, and NF 15d indicate samples naturally fermented for 5, 10, and 15 days, respectively. LF 5d, LF 10d, and LF 15d indicate samples fermented with *Lactobacillus plantarum* for 5, 10, and 15 days, respectively. FLF 5d, FLF 10d, and FLF 15d indicate samples fermented with flavourzyme and *Lactobacillus plantarum* for 5, 10, and 15 days, respectively.

the odor intensity of grass carp samples. Furthermore, the odor attribute intensity was noticeably higher in the FLF group samples than in the NF and LF groups samples, indicating that combined Flavourzyme and L. *plantarum* fermentation enhanced the aroma formation in grass carp due to protein degradation. This result was consistent with the findings of Yang, Jiang, et al. (2020), Yang, Liu, et al. (2020), who reported that adding Flavourzyme promoted higher volatile compound formation in fermented fish products, including alcohols, aldehydes, and esters. Furthermore, related research indicated that specific protein structures, such as hydrophobic pockets, amino acid side chains, and terminal ends, might contribute to flavor compound release and retention by interacting in specific configurations (Gu et al., 2020; Xue et al., 2021). The protein degradation due to Flavourzyme addition may reduce the binding capacity between protein and volatile compounds, facilitating the volatile compound release.

3.7. Sensory assessment

Fig. 4 shows the sensory evaluation results of the three groups during fermentation after the QDA test. The scores for the five sensory attributes increased progressively as the fermentation progressed, indicating that the comprehensive sensory performance of the samples in each fermented group displayed gradual improvement. Notably, the samples in the FLF group consistently scored higher than those in the other two groups in terms of color, odor, taste, texture, and overall acceptability, suggesting that Flavourzyme and L. *plantarum* fermentation promoted

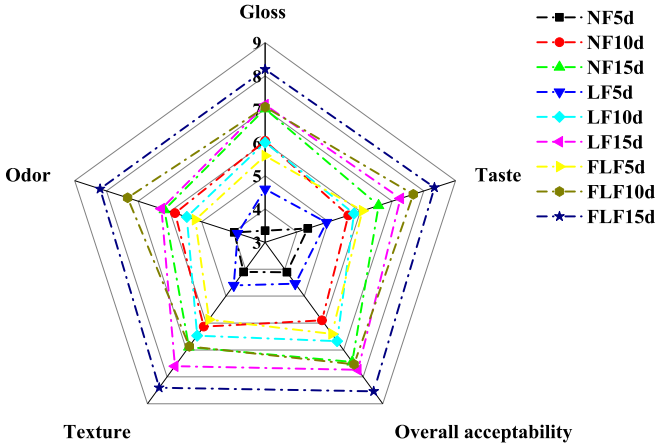

Fig. 4. Sensory evaluation analysis of fermented grass carp from NF, LF, and FLF groups during fermentation. NF 5d, NF 10d, and NF 15d indicate samples naturally fermented for 5, 10, and 15 days, respectively. LF 5d, LF 10d, and LF 15d indicate samples fermented with *Lactobacillus plantarum* for 5, 10, and 15 days, respectively. FLF 5d, FLF 10d, and FLF 15d indicate samples fermented with flavourzyme and *Lactobacillus plantarum* for 5, 10, and 15 days, respectively.

the development of superior sensory qualities in fermented grass carp products. Furthermore, the taste and odor of the FLF group received significantly higher scores compared with the other sensory attributes, indicating that Flavourzyme and L. *plantarum* fermentation substantially enhanced the mouthfeel and odor profile of the fermented grass carp products. These results were also confirmed by those obtained via the E-tongue and E-nose. At the end of fermentation (15 d), combined Flavourzyme and L. *plantarum* fermentation resulted in a ruddier color, a more intense acid fragrance and fermented flavor, a stronger sweet and umami taste, and an improved mouthfeel in the fermented grass carp products, which increased consumer acceptance.

4. Conclusion

This study examines the impact of Flavourzyme and L. *plantarum* on the flavor development in fermented grass carp by investigating the protein degradation and sensory evaluation indexes including the moisture content, TN, NPN, PI, FAAs, TCA-soluble peptides, E-tongue, E-nose, and sensory attributes. The NF and LF samples are regarded as the control groups. The results show that extending the fermentation period decreases the moisture content, protein degradation, and water-soluble flavor compound formation in the fermented grass carp products. Combined Flavourzyme and L. *plantarum* fermentation further increased moisture loss, facilitated protein hydrolysis, and promoted the production of TCA-soluble peptides and FAAs compared to the other two fermentation methods (Control groups). Consequently, adding Flavourzyme and L. *plantarum* significantly enhances the flavor and nutritional quality of the fermented grass carp samples. Additionally, the E-tongue and E-nose results indicated that the products fermented with Flavourzyme and L. *plantarum* exhibited stronger umami and sour taste and aroma. The sensory evaluation results demonstrate that combined fermentation substantially improved the sensory quality of the fermented samples, particularly in terms of odor and taste. Therefore, utilizing Flavourzyme and L. *plantarum* during the fermentation process can elevate the flavor quality of fermented grass carp products. This research may offer several theoretical bases for the improvement of fermentation recipes and the commercial manufacturing of excellent fermented fish products.

CRediT authorship contribution statement

Naiyong Xiao: Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Qiang Zhang:** Writing – review & editing, Visualization, Formal analysis. **Huiya Xu:** Writing – review & editing, Methodology, Conceptualization. **Changliang Zheng:** Writing – review & editing. **Yantao Yin:** Writing – review & editing. **Shucheng Liu:** Writing – review & editing, Funding acquisition. **Wenzheng Shi:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that no conflict of interest exist in this research article.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101439.

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