



## 酵母接种发酵对鳙鱼肉气味的影响

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**摘要:** 研究酿酒酵母发酵对鳙鱼肉气味的影响, 为鳙的加工提供一定的理论依据。实验以鳙鱼肉为对象, 以酿酒酵母作为发酵剂, 采用顶空-固相微萃取-气相色谱-质谱法(HS-SPME-GC-MS)结合电子鼻(E-nose)分别研究了在发酵3和5 d时的气味变化。结果显示, 经酿酒酵母发酵后, 鱼肉中的酯类物质增加了12种, 包括壬醛、癸醛、己酸乙酯、癸酸乙酯、油酸乙酯和甲酸甲酯等, 赋予了鱼肉水果香气和杏仁香气, 使风味物质更加丰富。此外, 经酿酒酵母发酵后, 鳙鱼肉中原本具有土腥味的物质1-辛烯-3-醇含量有所下降, 在3和5 d后分别减少了16.04%和18.09%, 极大地改善了鳙鱼肉的气味。电子鼻结合主成分分析, 不同处理鱼肉可以明显区分, 说明酵母发酵对鳙鱼肉气味影响较大。气味活性物质分析结果得出, 经酵母发酵3 d后的鱼肉主体气味物质有8种, 比未添加酵母发酵多3种, 表明酵母菌的添加增加了鳙的主体气味物质。不同处理组中的酮类及烃类物质, 如3-辛酮、2-庚酮、石竹烯、D-柠檬烯、蒈、壬酸乙酯、己酸乙酯、2-辛烯醇等, 对风味也有一定的贡献作用。

**关键词:** 鳙; 发酵; 酿酒酵母; 气味

中图分类号: TQ 926.4; TS 254.4

文献标志码: A

据统计, 2020年全国水产品总产量为6549.02万t, 比上年增长1.06%, 其中淡水水产品产量为3234.64万t, 淡水加工水产品411.51万t<sup>[1]</sup>。鳙(*Hypophthalmichthys nobilis*)属于淡水鱼类, 肉薄刺多、土腥味较重, 受消费者喜爱程度较低。部分鳙被加工成冻品鱼片、鱼粉制品及鱼丸, 市场上主要以活体的销售为主, 使用率不高, 还产生很多的废弃物, 污染环境, 资源浪费严重<sup>[2]</sup>。

近年来, 通过发酵改善鱼和肉制品的感官特性受到了极大的关注<sup>[3]</sup>。目前在鱼类发酵中使用最多的是乳酸菌, 其在发酵过程中会产酸降低pH来抑制其他腐败菌的生长, 并通过酸诱导蛋白质变性来增强质地特性、改善口感。如黄忠白等<sup>[4]</sup>

利用植物乳杆菌(*Lactobacillus plantarum*)发酵金枪鱼的精巢(鱼白), 得出经发酵后的鱼白中己醛、1-辛烯-3-醇等具有腥味的物质减少, 脱腥增香的效果明显。然而, 酵母菌作为另一种典型的食品工业益生菌, 可以产生令人愉快的果香味<sup>[5]</sup>, 但尚未广泛应用于鱼类产品加工中。Simoncini等<sup>[6]</sup>的研究表明, 酵母菌能产生各种高活性酶而不催化产生致癌物质, 可以保证产品的生物安全性。

挥发性风味物质通常被认为是食品风味质量的重要参数, 气味的好坏往往影响着淡水鱼的销售情况。目前用于挥发性物质分析的方法主要有气相色谱-质谱(GC-MS)<sup>[7]</sup>、液相色谱-质谱(LC-MS)<sup>[8]</sup>、顶空固相微萃取(HS-SPME)<sup>[9]</sup>、电子鼻(E-

收稿日期: 2021-04-07 修回日期: 2021-04-30

资助项目: 国家重点研发计划(2019YFD0902003)

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中国水产学会主办 sponsored by China Society of Fisheries

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nose) 技术等。顶空-固相微萃取-气相色谱-质谱法(HS-SPME-GC-MS)和主成分分析(PCA)的组合使用可以分析发酵过程中鱼肉挥发性物质的变化<sup>[10]</sup>。

本研究使用 HS-SPME-GC-MS 结合电子鼻分析酿酒酵母(*Saccharomyces cerevisiae*)发酵对鳙鱼肉气味的影响, 旨在改善其风味, 对发酵技术在淡水鱼类加工中的应用提供了一定的理论依据。

## 1 材料与方法

### 1.1 材料与试剂

鳙购于“叮咚买菜”(上海市昊坤国际大厦店), 平均体质量(1.5±0.2) kg, 去头、去内脏、去皮, 清水冲洗干净取背肉并切成3 cm×2 cm×2 cm(长×宽×厚)的鱼块。安琪酵母、食盐及白砂糖购自上海市浦东新区古棕路农工商超市。

### 1.2 仪器与设备

恒温恒湿培养箱(LHS-250SCN), 上海慧泰仪器制造有限公司; 电热鼓风干燥箱(DHG-9053A), 上海一恒科学仪器有限公司; 其他均为实验室常用仪器。固相微萃取(SPME)手动进样手柄, 65 μm PDMS/DVB(聚二甲基硅氧烷/二乙烯基苯)萃取头, 美国 Supelco 公司; FOX-4000 气味指纹分析仪(电子鼻, E-nose), 法国 AlphaM.O.S 公司; 6890 GC-5975 MS 联用仪, 美国 Agilent 公司。

### 1.3 样品的制备

将5%食盐和3%白砂糖均匀涂抹于鳙鱼块表面, 在4 °C下腌制2 h。取0 d的样品作为对照组, 记为0组。然后分为两组: 表面涂抹0.5%的酵母, 记为Y组; 表面不涂抹酵母, 记为Z组。将鱼块放入发酵罐中, 并置于28 °C、相对湿度(RH)85%的培养箱中, 取样时间分别为3和5 d, 分别记为Z3、Z5、Y3、Y5。

### 1.4 实验方法

**电子鼻分析** 参考Yang等<sup>[11]</sup>的方法并稍作修改, 准确称取2.5 g切碎的鱼肉, 加入5 mL 0.18% NaCl溶液, 匀浆后置于自动进样瓶中(规格: 10 mL)。电子鼻条件: 载气为干燥洁净的空气, 流速为150 mL/min; 样品温度4 °C, 清洗时间为120 s, 测样时间为600 s。顶空产生参数: 产生时间为600 s, 产生时间为600 s, 搅动速率500 r/min。顶空注射参数: 注射体积2 500 μL; 注射速率2 500 μL/s。每组样品重复测定8次。

**顶空固相微萃取** 利用HS-SPME-GC-MS法测定发酵鱼肉中的挥发性成分。向5 g切碎的鱼肉中加入10 mL 0.18% NaCl溶液, 匀浆后置于顶空进样瓶中(规格: 20 mL)。样品在水浴温度为50 °C下萃取40 min。然后将萃取头插入GC进样口, 250 °C解析5 min。

**GC-MS分析** GC-MS测定: HP-5MS弹性毛细管柱(30 m×0.25 mm, 0.25 μm); 不分流模式进样。升温程序参考Zang等<sup>[12]</sup>的方法, 略作修改: 柱初始温度为40 °C, 保持2 min, 以4 °C/min的速率升温至160 °C, 然后以10 °C/min的速率升温至250 °C, 保持5 min。质谱条件: 离子源温度为230 °C, 四极杆温度为150 °C, 电子能量70 eV。GC-MS数据使用Xcalibur软件分析。通过查询NIST和Wily质谱数据库确认定性, 为了保证被检测组分的可靠性, 根据现有文献, 当物质正反匹配度均大于800时(最大值1 000)才予以确认。使用面积归一化法确定不同样品中各挥发性化合物的相对百分含量<sup>[13]</sup>。每种挥发性化合物的贡献根据其相对气味活性值(relative odor activity value, ROAV)来描述<sup>[14]</sup>:

$$\text{ROAV} \approx \frac{\text{CR}_i}{\text{CR}_{\text{stan}}} \times \frac{T_{\text{stan}}}{T_i} \times 100$$

式中, CR<sub>i</sub>和CR<sub>stan</sub>分别指每种挥发性成分和对样品整体风味贡献最大的成分的相对百分比(%)含量。T<sub>i</sub>和T<sub>stan</sub>分别指文献中报道的每种挥发性化合物的水相和对整体风味贡献最大的成分的气味阈值。化合物的ROAV越大, 对整体风味的贡献就越大。一般认为ROAV≥1的物质是样品的主要挥发性化合物; 然而, ROAV为0.1~1的其他挥发性化合物也会影响样品的整体风味。

### 1.5 数据分析

使用Origin 8.5软件进行数据处理和作图, 利用SPSS 20软件进行数据统计分析。主成分分析(principal component analysis, PCA)由AlphaSoft V11自带统计软件进行分析。

## 2 结果

### 2.1 电子鼻结果分析

FOX4000中包含的18个传感器如表1所示<sup>[15]</sup>。图1为传感器对鱼肉气味感应后使用软件作出的雷达图, 电子鼻要求待测样品对传感器的最大响

**表 1 电子鼻传感器及相应的代表性敏感化合物**  
**Tab. 1 Electronic nose sensors and corresponding representative sensitive compounds**

传感器 sensors	敏感化合物 sensitive compounds
LY2/LG	氧氮化物, 硫化物, 氯化物, 氟 oxynitride, sulfde, chloride, fluorine
LY2/G	碳氧化物, 胺, 氨 carbon oxide, amines, ammonia
LY2/AA	氨, 乙醇, 丙酮 ammonia, ethanol, acetone
LY2/GH	胺, 氨 amines, ammonia
LY2/gC TL	硫化氢 hydrogen sulfde
LY2/gC T	丙烷, 丁烷 propane, butane
T30/1	氯化物 chloride
P10/1	碳氢化合物, 氨, 氯 hydrocarbon, ammonia, chlorine
P10/2	甲烷, 乙烷 methane, ethane
P40/1	氯, 氟 chlorine, fluorine
T70/2	甲苯, 二甲苯, 一氧化碳 toluene, xylene, carbon oxide
PA/2	胺, 氢氧化铵, 乙醇 amines, ammonium hydroxide, ethanol
P30/1	碳氢化合物, 氨, 乙醇 hydrocarbon, ammonia, ethanol
P40/2	硫化氢, 氯, 氟 hydrogen sulfde, chlorine, fluorine
P30/2	酮, 亚硫酸氢盐 ketone, hydrogen sulfde
T40/2	氯, 氟 chlorine, fluorine
T40/1	氟 fluorine
TA/2	乙醇 ethanol

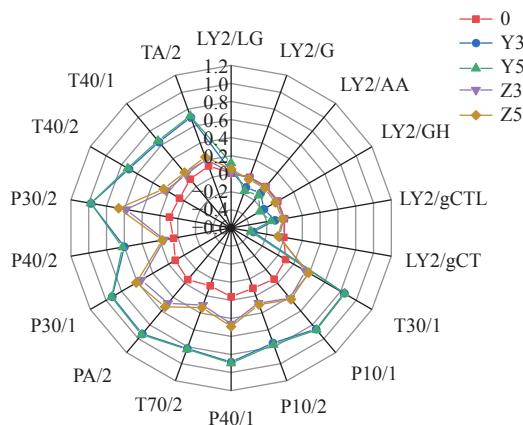


图 1 不同处理**鱠**鱼肉挥发性气味雷达图

Fig. 1 Radar chart of volatile odor of *H. nobilis* after different treatments

应大于 0.5, 本次实验的所有样品均达到了检测要求。通过雷达图可以直观地看出电子鼻对 0、Z 和 Y 组中鱼肉响应值的差异, 结合表 1 可以看出, 传感器对样品中的碳氢化合物、醇类及酮类成分比较灵敏。0、Z 和 Y 组样品在 TA/2、T40/1、T40/2、P30/2、P40/2、P30/1、PA/2、T70/2、P40/1、P10/2、P10/1 和 T30/1 等传感器中的信号响应值有明显的差异, 说明这 12 个传感器检测出的挥发性化合物组成相差较大, 而在其他传感器中的信号响应值几乎重合, 说明其余的传感器检测出的化合物组分相似。

主成分分析是一种多元统计方法, 它是将一组可能有相关性的变量通过正交变换的方法转为一组线性不相关变量<sup>[16]</sup>。图 2 为 0、Z 和 Y 组的电子鼻主成分分析图。图中显示了两个主要组件轴 (PC1 和 PC2), 这两个特征向量的累积方差贡献率越大, 反映的多指标信息越多<sup>[17]</sup>。其中 PC1 的贡献率为 96.983%, PC2 的贡献率为 2.801%, 总贡献率为 99.784%。Z 组和 Y 组数据点分布相差甚远, 表明接种和未接种酵母的鱼肉气味有较大的差异。Z 和 Y 组中, 0 和 3 d 的数据点分布较远, 表明气味差距较大; 而 3 和 5 d 的数据点分布较近, 表明气味差异不明显。但是, Z 组中的数据点分布在第二象限, Y 组中的数据点分布在第三象限, 说明 Z 和 Y 组气味有明显的差异。主成分中的辨别指数表明各样品间的差异程度, 正值表示可以利用主成分分析区分, 越大区分程度越好。

## 2.2 GC-MS 分析

不同处理组**鱠**鱼肉中的挥发性物质主要包括醛酮类(表 2)、醇类、酯类(表 3)、烃类及其他化合物(表 4), 其中 Z 组中醇类物质相对含量占比

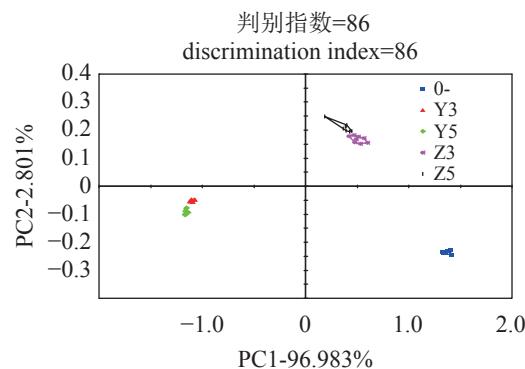


图 2 不同处理**鱠**鱼肉 PCA 分析图

Fig. 2 PCA analysis chart of *H. nobilis* after different treatments

表2 不同组中醛酮类物质的相对含量  
Tab. 2 Relative content of aldehydes and ketones in different groups %

名称 names	组别 groups				
	0	Z3	Z5	Y3	Y5
己醛 hexanal	24.95±0.05 <sup>c</sup>	20.66±0.07 <sup>d</sup>	13.38±0.11 <sup>c</sup>	3.77±0.03 <sup>a</sup>	2.21±0.05 <sup>b</sup>
壬醛 nonanal	5.78±0.09 <sup>c</sup>	5.01±0.05 <sup>d</sup>	3.75±0.04 <sup>b</sup>	4.49±0.08 <sup>c</sup>	1.65±0.04 <sup>a</sup>
庚醛 enanthal	0.64±0.01 <sup>b</sup>	0.16±0.03 <sup>a</sup>	—	—	—
癸醛 decanal	1.67±0.02 <sup>b</sup>	—	9.87±0.18 <sup>d</sup>	2.06±0.03 <sup>c</sup>	0.90±0.02 <sup>a</sup>
十六烷烯醛 hexadecenal	—	14.24±0.05	—	—	—
十四烷醛 tetradecanal	—	1.52±0.02 <sup>a</sup>	8.54±0.05 <sup>b</sup>	—	—
顺-4-癸醛 cis-4-decanal	—	—	3.45±0.05 <sup>a</sup>	3.63±0.03 <sup>b</sup>	—
苯乙醛 phenylacetaldehyde	—	—	—	—	2.94±0.06
2-十一烷酮 2-undecanone	2.64±0.04 <sup>c</sup>	2.54±0.04 <sup>b</sup>	2.37±0.02 <sup>a</sup>	—	—
2,3-辛二酮 2,3-octanedione	2.76±0.06 <sup>b</sup>	—	—	—	0.68±0.02 <sup>a</sup>
3-辛酮 3-octanone	—	4.13±0.02	—	—	—
2-壬酮 2-nonanone	—	1.70±0.02 <sup>a</sup>	2.23±0.02 <sup>b</sup>	—	—
2-庚酮 2-heptanone	—	—	2.53±0.07	—	—
麝香酮 muskone	—	—	—	—	0.80±0.02
总计 total	38.44	49.96	46.12	13.95	9.18

注：同行不同上标字母表示差异显著 ( $P<0.05$ )；“—”表示未检出，下同  
Notes: different superscript letters in the same line indicate significant differences ( $P<0.05$ ); “—” means not detected, the same below

较高；Y组中酯类物质相对含量占比较高。Y3和Y5组的挥发性化合物各有28和30种，比Z3和Z5组各多出6种，说明利用酵母发酵可以丰富鱼肉的气味。

### 2.3 气味活性物质分析

各挥发性化合物的ROAV值是结合挥发性成分的阈值<sup>[18-21]</sup>和各物质的相对含量计算而来。发酵鱼的主体气味物质ROAV见表5。1-辛烯-3-醇在各组中的相对含量较高，阈值较低，定义其 $ROAV_{stan}=100$ 。Z3组中 $ROAV>1$ 的主体气味物质有5种，Y3组中 $ROAV>1$ 的物质有8种，表明添加酵母可以丰富鳙鱼肉的风味。Z组和Y组中 $0.1\leq ROAV \leq 1$ 的物质主要有3-辛酮、2-庚酮、石竹烯、D-柠檬烯、萘、壬酸乙酯、己酸乙酯和2-辛烯醇，它们对鱼肉的风味也有一定的作用。

## 3 讨论

醇类主要由脂质氧化产生，它在鱼肉发酵的风味贡献中起着重要的作用<sup>[22]</sup>。1-辛烯-3-醇通常被描述为具有蘑菇味、青草味和泥土味的土腥味物质，酵母的添加使其含量有所下降，这与苏怡等<sup>[23]</sup>的研究结果相似，并且它被认为是亚油酸或

其他多不饱和脂肪酸的氧化产物<sup>[19]</sup>。同样被认为具有青草味的1-己醇含量在Y3和Y5组中分别下降了6.41%和6.93%，说明酵母的添加可以改善鳙鱼肉的风味。此外，直链醇中2-庚醇具有较低的阈值，可以提供香味。醛被认为是发酵肉风味形成的必需化合物，因为醛能散发出青草、坚果、糖果和奶酪味，并且具有低阈值<sup>[22]</sup>。己醛、壬醛和癸醛是样品中的主要气味物质，这些直链醛源自不饱和脂肪酸的氧化，是微生物酶的产物<sup>[24]</sup>。Z组中的十四烷醛被认为具有酱油风味<sup>[25]</sup>。

酵母菌在发酵过程中会催化产生酯类物质，大多数酯类物质会散发出果香<sup>[21]</sup>。酯类挥发性物质含量的增加可以归因于有机酸类与醇类的酯化作用，这种酯化作用主要由酵母发酵过程中产生的酯化酶引起<sup>[26]</sup>。由短链酸产生的酯具有水果香气，而由长链酸产生的酯具有脂肪气味<sup>[27]</sup>。Y组在发酵过程中产生的酯类有12种，包括1,2-苯二甲酸丁辛酯、辛酸乙酯、己酸乙酯、癸酸乙酯、十四烷酸乙酯、9-十六碳烯酸乙酯、十六酸乙酯、壬酸乙酯、十二酸乙酯、5,8,11,14-二十碳四烯酸乙酯、油酸乙酯和甲酸甲酯等。其中，己酸乙酯被Oliveira等<sup>[28]</sup>认为具有苹果皮、菠萝和草莓香气。Z组和Y组中均有酮类物质被检出，Olesen



表5 不同组别之间鳙鱼肉挥发性气味成分的相对气味活度值(ROAV)

Tab. 5 ROAV of volatile odor components of *H. nobilis* meat in different groups

化合物名称 names	阈值/(μg/kg) threshold	组别 groups				
		0	Z3	Z5	Y3	Y5
1-辛烯-3-醇 1-octene-3-ol	1	100	100	100	100	100
2-庚醇 2-heptanol	10.87	—	0.94	1.65	—	—
1-己醇 1-hexanol	5.6	7.33	—	3.13	7.96	10.29
2-辛烯醇 2-octenol	40	—	—	0.31	0.91	—
壬醛 nonanal	1	27.38	24.89	21.43	88.56	54.64
己醛 hexanal	4.5	26.26	22.81	16.99	16.52	16.26
癸醛 decanal	2	3.96	—	28.20	20.32	14.90
庚醛 enanthal	3	1.01	0.26	—	—	—
苯乙醛 phenylacetaldehyde	4	—	—	—	—	24.34
3-辛酮 3-octanone	28	—	0.73	—	—	—
2-壬酮 2-nonanone	5	—	1.69	2.55	—	—
2-庚酮 2-heptanone	140	—	—	—	0.10	—
2-十一烷酮 2-undecanone	7	1.79	1.80	1.93	—	—
石竹烯 caryophyllene	40	—	0.19	0.28	1.53	—
D-柠檬烯 D-limonene	10	—	—	—	0.38	—
萘 naphthalene	60	0.18	—	—	0.57	1.19
丁香酚 eugenol	6	1.80	—	—	46.29	—
辛酸乙酯 ethyl caprylate	19.30	—	—	—	9.40	17.40
壬酸乙酯 ethyl nonanoate	3000	—	—	—	—	<0.1
己酸乙酯 ethyl caproate	320.40	—	—	—	<0.1	0.14

等<sup>[29]</sup>认为很多酮类主要来源于饱和脂肪酸的β-氧化和发酵过程中氨基酸的降解。其中,2,3-辛二酮赋予发酵鱼果香和油脂的气味。有研究表明C8~C19烷烃无味且具有较高的阈值,对整体风味没有实际贡献作用,是由脂肪酸氧化或类胡萝卜素分解引起的<sup>[30-31]</sup>。醇类、醛类、酯类及烃类等挥发性化合物共同作用使得发酵鱼具有独特的风味。

利用HS-SPME-GC-MS结合电子鼻技术分析经酵母发酵后鳙鱼肉气味的变化。电子鼻结果显示发酵和未发酵的鱼肉气味差异明显。酵母菌接种发酵可以改善鱼肉的气味,使鱼肉中原本含量较高且具有土腥味的1-辛烯-3-醇、己醛等不愉快的气味有所减少;并且鱼肉中的酯类化合物增加了12种,其中己酸乙酯、癸酸乙酯、油酸乙酯、辛酸乙酯和甲酸甲酯等赋予鱼肉水果香气、杏仁味和酒香气等。本研究对发酵技术在淡水鱼类加工中的应用提供了一定的理论依据。

(作者声明本文无实际或潜在的利益冲突)

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## Effects of yeast inoculation and fermentation on the odor of *Hypophthalmichthys nobilis*

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**Abstract:** Studying the effect of yeast fermentation on the odor of *Hypophthalmichthys nobilis* can provide a certain theoretical basis for the processing of the fish. *H. nobilis* thin thorns and more meat and a strong earthy smell, which is less popular with consumers. Some of them are processed into frozen fish fillets, fish meal products and fish balls. The market is mainly for live sales, the utilization rate is not high, and a lot of waste is generated, which pollutes the environment and wastes resources. In recent years, the improvement of the organoleptic properties of fish and meat products through fermentation has received considerable attention. Traditional fermented fish is fermented under natural conditions by using microorganisms carried by the environment or by itself. The fermentation process is difficult to control and it is difficult to ensure that the number and types of microorganisms are the

same, and the quality of fermented products varies greatly; furthermore, the product fermentation cycle is longer, resulting in fish meat the excessive oxidation of fatty acids reduces the nutritional quality of processed products. In addition, traditional fermented fish methods are diverse, processing environments vary greatly, product quality is unstable, and fermented flavors vary greatly. Raw materials, microbial activities, environment and other factors will affect the quality of fermented fish. The core technology is the breeding of microbial starters. Microbes play an important role in the physical, chemical, nutritional and sensory properties of fermented products. Therefore, research and development of related microbial starters to improve the quality of fermented fish products has practical significance. Appropriate processes are sought to improve the flavor quality characteristics of the products. At present, lactic acid bacteria are the most used in fish fermentation, because they produce acid during the fermentation process to reduce the pH value to inhibit the growth of other spoilage bacteria, and through acid-induced protein denaturation to enhance texture characteristics and improve taste. However, yeast, as another typical food industry probiotic, can produce more pleasant fruit aromas, but it has not been used in industrial fish products. This research uses silver carp as the research object, *Saccharomyces cerevisiae* as the fermenting agent, and combines headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) with electronic nose (E-nose) to study the odor changes during 3 and 5 days of fermentation. Results showed that after yeast fermentation, there were 12 kinds of esters, including nonanal, decanal, ethyl caproate, ethyl caproate, ethyl oleate and methyl formate, which gave the fish a 'fruity aroma' and 'almond aroma', so that the flavor substances were more abundant. After fermentation, the content of 1-octen-3-ol decreased by 16.04% and 18.09% after 3 and 5 days, which greatly improved the odor of *H. nobilis*. The E-nose and principal component analyses found that the different groups could be discriminated easily, indicating that the smell of the fish after the *S. cerevisiae* fermentation has changed greatly. The analysis of odor substance activity showed that there were 8 main odor substances after 3 days of fermentation, which was 3 more than that the group without adding *S. cerevisiae*, indicating that add *S. cerevisiae* can increase the main odor substances of silver carp. Ketones and hydrocarbons in different treatment groups also contributed to flavor, such as 3-octanone, 2-heptanone, caryophyllene, D-limonene, naphthalene, ethyl nonanoate, ethyl caproate, 2-octenol, etc.

**Key words:** *Hypophthalmichthys nobilis*; fermentation; *Saccharomyces cerevisiae*; flavor

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**Funding projects:** National Key R & D Program of China (2019YFD0902003)