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仿刺参性腺酶解过程风味变化*

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摘要 生物酶解是提高水产品蛋白质利用率的有效手段，且酶解过程往往伴随肽类、氨基酸、小分子挥发性成分等的生成或反应，进而引起酶解液风味变化。仿刺参(*Apostichopus japonicus*)性腺富含蛋白质和多种功效成分，具有良好的开发利用前景。为探究仿刺参性腺酶解过程中蛋白质和风味的变化规律，采用中性蛋白酶对其进行酶解，对酶解过程中可溶性蛋白质、氨基酸态氮、游离氨基酸组成、挥发性成分的变化情况进行了检测分析。结果显示，仿刺参性腺匀浆液中可溶性蛋白质和氨基酸态氮的初始含量分别为 1.14 和 0.15 g/100 g，可溶性蛋白质在酶解前 30 min 内迅速增加，之后基本保持不变，氨基酸态氮含量在前 90 min 内随时间延长而逐渐增加，90 min 后略有下降，90 min 时水解度最大，达 43.66%。随着酶解时间的延长，酶解液的鲜味有所增强，腥味减弱，甜味和苦味也略有增强。酶解后游离氨基酸含量显著增加(P<0.05)，其中，呈鲜味的谷氨酸含量最高，其次为呈甜味的甘氨酸和丙氨酸。仿刺参性腺酶解过程气味发生明显变化，烃类种类和相对含量均明显增加，二甲基硫醚含量显著降低，这可能是酶解液腥味减弱的主要原因。

关键词 海参性腺；水解度；电子鼻；GC-MS；风味

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海参属于棘皮动物门(Echinodermata)、海参纲(Holothuroidea)，全世界海参约 900 多种，其中 40 种可供食用(廖玉麟, 2001)。中国是世界上海参养殖产量最大的国家，2020 年产量达到 196 564 t (农业农村部渔业渔政管理局, 2021)，养殖种类为仿刺参(*Apostichopus japonicus*)，养殖区域集中在辽宁、山东、福建等地。仿刺参是我国药食同源的典型品种，具有极高的食用和经济价值。

海参具有自溶的特点，活海参采捕上岸后，必须立即进行加工，加工过程会产生 30%左右的副产物，如海参肠、性腺、呼吸树等组织，目前，对这些副产物加工利用的程度很低，有些甚至被直接丢弃，造成环境污染和资源浪费(Mamelona *et al*, 2010)。海参性腺富含优质蛋白(Sun *et al*, 2017)，可用于开发膳食补充剂型产品。海参性腺还含有海参多糖、海参皂苷、活性脂质等多种活性成分(Yang *et al*, 2020; 袁文鹏等, 2010; 张健等, 2013)。Mamelona 等(2007)发现，大西洋海参性腺中酚类和黄酮类物质含量较高，是潜在的天然抗氧化剂来源。太平洋一些地区，如新西兰和库克群岛的居民定期从海参内脏中收集性腺，用于

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烹饪美食或作为传统膳食的蛋白质来源(Drumm *et al*, 2005)。在日本，海参性腺被加工成丸剂和粉剂功能性产品，价格昂贵(曹荣等, 2020)，而我国对海参性腺的开发利用程度还很低。

海参性腺蛋白含量高，多种成分具有生理活性(刘昕等, 2016)，极具开发潜力。生物酶法是提高蛋白质利用率的有效手段，可以将大分子蛋白降解为易溶解且有利于人体吸收的小分子肽类和氨基酸，同时，在一定程度上缓解原料腥味重的问题(Wang *et al*, 2018; Li *et al*, 2016)。本研究以仿刺参性腺为原料，采用前期研究确立的酶解条件(曹荣等, 2012)对仿刺参性腺进行酶解，对酶解过程中可溶性蛋白、氨基酸态氮含量变化进行检测分析，对风味变化进行研究，以期为仿刺参性腺相关产品的开发提供参考。

1 材料与方法

1.1 实验材料与设备

仿刺参性腺由山东青岛海滨食品有限公司提供，系当年春季采捕底播仿刺参时现场分离得到；中性蛋白酶(20 万 U/g)、牛血清白蛋白、硫酸铜、酒石酸钠钾、三氯乙酸、冰乙酸、乙酸钠、甲醛、乙酰丙酮和硫酸铵等均为国产分析纯，购自国药集团化学试剂有限公司。

SHA-B 型恒温震荡水浴锅(江苏常州国华电器有限公司)；Centrifuge 5804r 型冷冻离心机(上海富众生物科学有限公司)；UV-2800 型紫外可见分光光度计(上海尤尼克柯仪器有限公司)；PEN3 便携式电子鼻(AIRSENSE 公司，德国)；L-8900 型氨基酸自动分析仪(日立高新技术公司，日本)；7980A/5975C 型气相色谱–质谱联用仪(Agilent 公司，美国)。

1.2 实验方法

1.2.1 *仿刺参性腺酶解液的制备* 取新鲜仿刺参性腺匀浆，按料液比 1∶3 (*m*/*v*)混匀，加入中性蛋白酶 2000 U/g，在 45℃下酶解 2 h (曹荣等, 2012)，酶解过程每 30 min 取一次样，进行各项指标测定。

1.2.2 *可溶性蛋白含量测定* 取 5.0 mL 酶解液加入等体积的 TCA 溶液(质量分数为 15%)，静置 20 min 后，10 000 r/min 离心 10 min。上清液定容至 10 mL，取 1 mL 待测液，采用双缩脲比色法(王永华, 2010)测定可溶性蛋白质含量。

1.2.3 *氨基酸态氮含量测定* 参照 GB/T 5009.39-2003，采用甲醛比色法测定。

1.2.4 *水解度计算* 水解度 DH (degree of hydrolysis)按照下式(曹荣等, 2014)进行计算：

$$\mathrm{DH}(\%)=\frac{P_2-P_1+(N_2-N_1)\times 9.14}{N_0-P_1-N_1\times 9.14}\times 100$$

式中，P_2 为酶解液中可溶性蛋白质含量(mg/g)；P_1 为水解前匀浆液中可溶性蛋白质含量(mg/g)；N_2 为酶解液中氨基酸态氮含量(mg/g)；N_1 为水解前匀浆液中氨基酸态氮含量(mg/g)；N_0 为蛋白质总含量(mg/g)；9.14 为氨基酸态氮与氨基酸之间的换算系数，以 20 种氨基酸平均分子量 128 Da 计。

1.2.5 *游离氨基酸组成测定* 采用氨基酸自动分析仪测定游离氨基酸组成。

1.2.6 *挥发性成分测定* 采用 PEN3 电子鼻分析酶解液的气味特征。取 1 mL 酶解液置于顶空瓶中，孵育 15 min，准备时间 10 s，检测时间 60 s，清洗时间 60 s。电子鼻测定结果利用 WinMuster 进行线性判别分析(Linear Discriminant Analysis, LDA)。

采用 GC-MS 对酶解过程挥发性成分的相对含量进行进一步分析。气相色谱条件：HP-INNOWAX 毛细管色谱柱(30.00 m×250.00 μm×0.25 μm)，325℃，载气为 He，分流进样模式，分流比 5∶1，分流流量为 5 mL/min，恒压为 48 kPa，进样量为 20 μL，起始温度为 40℃，保持 5 min，以 8℃/min 升到 250℃，保持 5 min。质谱条件：离子源温度为 230℃，四极杆温度 150℃，质量扫描范围 30~400 u，电子能量为 70 eV。通过计算机检索谱库 NIST 08 进行定性分析，按照峰面积归一化法计算相对含量。

1.2.7 *感官评价* 将样品随机编号，由 6 位接受过感官评定培训的人员组成评定小组，按表 1 标准分别对海参性腺酶解液风味进行评分，结果以平均值表示。

1.2.8 *数据处理* 实验重复 2 次，每次设 3 个平行样品，结果以平均值±标准差(Mean±SD)表示，使用 Origin 软件对数据进行统计学处理，采用 *t*-test 进行组间差异显著性分析，$P<0.05$ 则差异显著。

2 结果与分析

2.1 仿刺参性腺酶解过程可溶性蛋白质、氨基酸态氮含量与水解度的变化

仿刺参性腺酶解过程可溶性蛋白质、氨基酸态氮含量变化如图 1a 所示。匀浆液中可溶性蛋白质初始含量为 1.14 g/100 g，在前 30 min 内迅速增加至 4.80 g/100 g 左右，之后基本保持不变。氨基酸态氮含量在前 90 min 内随时间延长而逐渐增加，初始匀浆液中氨基酸态氮含量为 0.15 g/100 g，90 min 时达

表 1　酶解液的风味感官评价标准

Tab.1　Sensory evaluation standard for flavor of enzymatic hydrolysate

风味特征 Flavor characteristics	特征描述 Flavor description	分值 Scores
鲜味 Fresh	鲜味浓厚，怡人 Stronger	5
	鲜味较明显，易察觉 Strong	4
	鲜味一般，可察觉 Average	3
	鲜味较淡，不易察觉 Light	2
	无特征鲜味 Not obvious	1
苦味 Bitter	苦味极重 Stronger	5
	苦味较重 Strong	4
	苦味一般 Average	3
	苦味较淡 Light	2
	无苦味 Not obvious	1
咸味 Salty	咸味极重 Stronger	5
	咸味较重 Strong	4
	咸味一般 Average	3
	咸味轻微 Light	2
	无咸味 Not obvious	1
甜味 Sweet	甜味极重 Stronger	5
	甜味较重 Strong	4
	甜味一般 Average	3
	甜味轻微 Light	2
	无甜味 Not obvious	1
腥味 Fishy	腥味极重 Stronger	5
	腥味重，难以忍受 Strong	4
	腥味一般，可忍受 Average	3
	腥味较淡 Light	2
	无腥味 Not obvious	1

到 0.50 g/100 g，之后虽略有下降，但差异并不显著(P>0.05)。水解度是衡量蛋白质水解程度的指标，可通过酶解过程中可溶性蛋白质和氨基酸态氮的含量变化表示。如图 1b 所示，水解度随酶解时间延长呈上升趋势，60 min 后趋于稳定，90 min 时水解度达 43.66%，120 min 时水解度略有下降，但与酶解 60 min 和 90 min 的水解度相比无显著差异(P>0.05)。苏明月等(2019)分析了牡蛎肉的酶解过程，发现可溶性蛋白质和水解度都呈先增加后基本保持不变的趋势，本研究结果与之基本一致。

2.2　仿刺参性腺酶解液的感官评价结果

以水产品加工副产物为原料进行酶解，得到的酶解产物往往风味较差(李利敏等, 2014)。随着酶解的进行，大分子蛋白质被降解为小分子肽类以及氨基酸。一些特定的肽类和氨基酸具有呈味特性，从而影响酶解液的风味特征。与此同时，伴随酶解过程，会生成一些小分子挥发性化合物(王红丽等, 2018)，影响酶解液的气味。

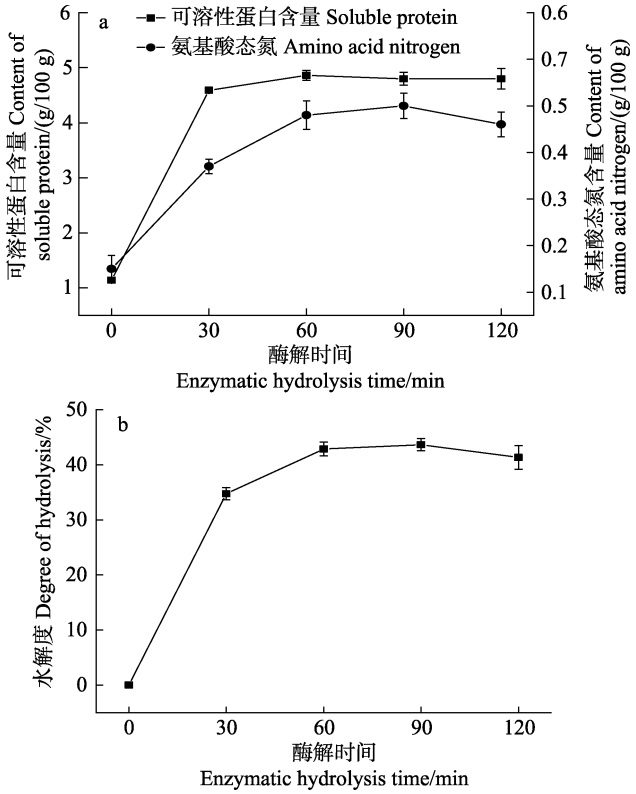

图 1　仿刺参性腺酶解过程可溶性蛋白质、氨基酸态氮含量(a)与水解度(b)变化情况

Fig.1　Changes in soluble protein, amino acid nitrogen (a) and degree of hydrolysis (b) of *A. japonicus* gonads during enzymatic hydrolysis

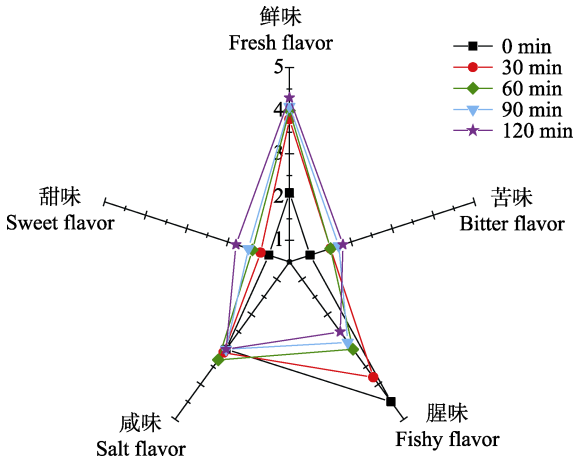

图 2　仿刺参性腺酶解过程感官评分

Fig.2　Sensory evaluation of *A. japonicus* gonad during enzymatic hydrolysis

仿刺参性腺酶解液的感官评分结果如图 2 所示。随着酶解时间延长，酶解液的鲜味有所增强，可能与蛋白质降解生成了较多呈鲜味的氨基酸有关，且酶解液咸味突出，可能会与鲜味产生相乘作用。赵阳等(2015)研究紫贻贝(*Mytilus edulis*)蛋白酶解过程中呈

味物质释放规律时发现，酶解过程中产生的肽类物质对酶解液风味有一定影响，主要是酶解过程中产生的寡肽与其他呈味物质发生相乘作用，使酶解液风味饱满、口感协调。随着酶解时间的延长，酶解液的腥味有所减弱，表明生物酶解在脱除不良风味方面有一定作用。另外，甜味和苦味也略有增强。

2.3 仿刺参性腺酶解过程中游离氨基酸的变化

游离氨基酸是重要的滋味成分。仿刺参性腺酶解过程游离氨基酸的变化情况见表 2。随酶解时间的延长，游离氨基酸含量逐渐增多。初始匀浆液中游离氨基酸总量为(725.07±33.12) mg/L，其中谷氨酸含量最高，为(270.56±9.78) mg/L；酶解 60 min 时，游离氨基酸含量为(2221.04±89.91) mg/L，其中谷氨酸含量仍为最高，甘氨酸和丙氨酸含量次之；酶解 120 min 时，游离氨基酸含量达(3277.49±131.02) mg/L，谷氨酸含量为(711.97±34.12) mg/L，其次为甘氨酸和丙氨酸。

谷氨酸是典型的鲜味氨基酸(Je *et al*, 2005)，酶解 120 min 时滋味活度值(Taste activity values, TAV)由初始的 5.41 增加至 14.24，对酶解液风味有重要贡献。赵谋明等(2006)对低值鱼酶解液中游离氨基酸进行了测定，认为谷氨酸、天冬氨酸与各种盐、有机酸的相乘作用可能对酶解液的呈味产生积极作用。仿刺参性腺酶解液中除谷氨酸外，其他种类氨基酸的 TAV 均小于 1，对滋味的贡献小。其中，丙氨酸的 TAV 值相对较高，达 0.77，赋予酶解液轻微甜味。在整个酶解过程中，蛋氨酸、亮氨酸和苯丙氨酸等苦味氨基酸尽管含量显著增加，但由于其阈值较高，对酶解液的滋味贡献值较低，说明酶解液中苦味并非由氨基酸产生，推测可能是酶解过程中生成了一些苦味肽。

2.4 仿刺参性腺酶解过程电子鼻分析结果

采用电子鼻分析仿刺参性腺酶解过程气味变化，结果见图 3。横坐标第一主成分的贡献率为 84.64%，纵坐标第二主成分的贡献率为 12.53%，总贡献率为 97.17%，基本涵盖了样本的信息。LDA 是将样品信号数据通过运算法则投影到某一方向，进而依据距离远近分辨组间气味差异大小。由图 3 可以看出，不同酶解时间的仿刺参性腺酶解液气味有明显差异，表明酶解过程伴随小分子挥发性化合物的生成或反应。

表 2 仿刺参性腺酶解过程中游离氨基酸含量的变化

Tab.2 Changes in free amino acids of *A. japonicus* gonad during enzymatic hydrolysis

氨基酸 Amino acids	呈味特征 Taste	阈值 Threshold /(mg/L)	酶解 0 min Hydrolysis for 0 minute		酶解 60 min Hydrolysis for 60 minutes		酶解 120 min Hydrolysis for 120 minutes	
			含量 Content /(mg/L)	TAV	含量 Content /(mg/L)	TAV	含量 Content /(mg/L)	TAV
天冬氨酸 Asp	鲜 Fresh	1000	24.24±0.87^{c}	0.02	74.93±2.45^{b}	0.07	87.43±2.78^{a}	0.09
谷氨酸 Glu	鲜 Fresh	50	270.56±9.78^{c}	5.41	559.61±26.65^{b}	11.19	711.97±34.12^{a}	14.24
丝氨酸 Ser	甜 Sweet	1500	18.25±0.55^{c}	0.01	101.64±4.45^{b}	0.07	129.15±5.93^{a}	0.09
甘氨酸 Gly	甜 Sweet	1300	164.88±6.45^{c}	0.13	399.51±18.22^{b}	0.31	521.05±21.71^{a}	0.40
组氨酸 His	苦 Bitter	200	1.68±0.04^{c}	0.01	19.47±0.78^{b}	0.10	31.26±1.25^{a}	0.16
精氨酸 Arg	苦/甜 Bitter/Sweet	500	22.41±0.57^{c}	0.04	140.92±7.43^{b}	0.28	218.95±11.28^{a}	0.44
苏氨酸 Thr	甜 Sweet	2600	26.78±0.91^{c}	0.01	123.49±6.12^{b}	0.05	170.46±6.13^{a}	0.07
丙氨酸 Ala	甜 Sweet	600	117.78±1.12^{c}	0.20	351.23±15.35^{b}	0.59	463.56±23.17^{a}	0.77
脯氨酸 Pro	苦/甜 Bitter/Sweet	3000	16.81±0.56^{c}	0.01	103.26±4.41^{b}	0.03	202.00±9.94^{a}	0.07
酪氨酸 Tyr	苦 Bitter	–	0.74±0.02^{c}	–	12.62±5.60^{b}	–	48.83±1.69^{a}	–
缬氨酸 Val	苦 Bitter	400	12.67±0.61^{c}	0.03	85.51±2.17^{b}	0.21	147.61±8.38^{a}	0.37
蛋氨酸 Met	苦 Bitter	300	1.63±0.05^{c}	0.01	32.81±1.19^{b}	0.11	89.05±2.10^{a}	0.30
异亮氨酸 Ile	苦 Bitter	900	1.78±0.06^{c}	0.00	17.01±0.57^{b}	0.02	55.88±1.16^{a}	0.06
亮氨酸 Leu	苦 Bitter	1900	3.05±0.15^{c}	0.00	52.72±2.27^{b}	0.03	187.05±5.58^{a}	0.10
苯丙氨酸 Phe	苦 Bitter	900	0.55±0.01^{c}	0.00	12.83±0.23^{b}	0.01	26.4±0.62^{a}	0.03
赖氨酸 Lys	苦/甜 Bitter/Sweet	500	41.27±1.80^{c}	0.08	133.49±5.31^{b}	0.27	186.86±4.88^{a}	0.37
合计 Total			725.07±33.12^{c}		2221.04±89.91^{b}		3277.49±131.02^{a}	

注：“–”表示未查到该阈值。同一行肩标不同字母表示差异显著(P<0.05)。

Note: “–” indicates that the threshold is not found. Different letters of shoulder marks in the same line showed significant difference (P<0.05).

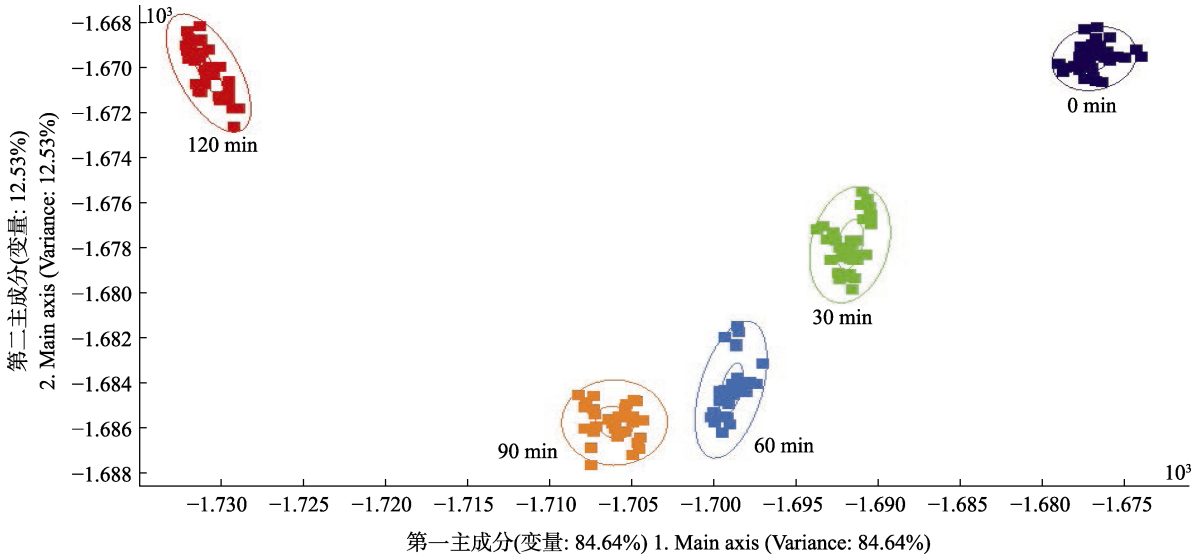

图 3　仿刺参性腺酶解液气味组成 LDA 结果

Fig.3　The LDA results of odor composition in *A. japonicus* gonad enzymatic hydrolysate

2.5　GC-MS 分析结果

采用 GC-MS 对仿刺参性腺酶解过程挥发性成分进行分析，结果见表 3。从仿刺参性腺酶解液中共鉴定出挥发性物质 43 种，其中，烃类 17 种、醇类 9 种、醛类 7 种、酮类 4 种以及其他 6 种。酶解 0、60 和 120 min 时，3 组样品中均含有的挥发性成分有 10 种，包括烃类 4 种、醇类 2 种、醛类 2 种、酮类 1 种和二甲基硫醚。初始匀浆液中共鉴定出 24 种挥发性成分化合物，其中，二甲基硫醚、1-辛醇和 1,5-二甲基-6-亚甲基螺环[2.4]庚烷含量相对较高。酶解 60 min 时，1-辛醇、1,5-二甲基-6-亚甲基螺环[2.4]庚烷比例增加，而二甲基硫醚相对含量显著降低，此时出现了一些新的挥发性成分，如环癸烷、环辛烯、苯乙醇、3-甲基-1-丁醇、乙酸。酶解 120 min 时，检测出对二甲苯、2-十二烯醛、苯乙醛、3-甲基丁醛、庚醇、Z-2-十八烯-1-醇、3-乙烯基-环辛烯、3-庚烯、1-甲基-4-亚甲基环己烷等挥发性成分，烃类相对含量从 31.77%增加到 48.49%。

部分烷烃类源于脂肪酸降解，芳香烃则主要由带芳香基的游离氨基酸氧化产生(Wang *et al*, 2020; Xie *et al*, 2008)。烷类和烃类风味特征为清香香甜型，但由于烷烃类阈值较高，对风味贡献不大(Martins *et al*, 2000)。烯烃类化合物具有较低的阈值，且大多带有果香味(赵玲等, 2021)，酶解 120 min 后烯烃含量有所增加，赋予酶解液一定的清新和香甜味。

醇类物质通常是由多不饱和脂肪酸氧化产生。饱和醇类化合物阈值较高，对风味贡献不大。不饱和的烯醇式结构化合物阈值较低，具有独特的清香、果香、花香和甜味等令人愉悦的风味，对酶解液风味有正面作用(Fratini *et al*, 2012)。1-辛醇的相对含量由初始的 19.78%增加至 29.35%，为酶解液增添了柑橘和玫瑰气息(步营等, 2020)。1-辛烯-3-醇是一种亚油酸的分解产物，表现出类似蘑菇的香气(Wu *et al*, 2014; Zhang *et al*, 2019)。乙醇是食品中重要的挥发性成分，主要来源于多不饱和脂肪酸的脂氧合反应(Radovcic *et al*, 2016)，其相对含量在酶解过程中逐渐降低。酶解前 60 min 醇类相对含量升高，主要与多不饱和脂肪酸的氧化有关；酶解 120 min 后，醇类含量有所降低，而醛类物质含量增多，可能与醇类物质发生氧化有关(Zou *et al*, 2018)。

酮类物质生成与氨基酸分解、多不饱和脂肪氧化降解等有关(徐永霞等, 2021)。酮类化合物的阈值一般比较高，对风味贡献不大。酮类物质中 3-羟基-2-丁酮变化最为明显，相对含量随酶解时间延长而增加。

醛类在高浓度时气味令人不悦，而低浓度时往往呈现出香气(高瑞昌等, 2013)。醛类物质主要来源于脂肪的氧化或氨基酸的 Strecker 降解反应，其中，6~9 个碳原子的醛一般具有清香、果香和脂肪香味，且阈值较低又具有叠加效应(周益奇等, 2006)，往往在食品风味中起重要作用。酶解后产生的苯乙醛可产生青草香或花香(Zhao *et al*, 2017)，3-甲基丁醛有轻微甜桔香或脂肪香气，主要衍生自脂质的温和氧化并可能赋予酶解液麦芽或坚果味(李学鹏等, 2020)。辛醛主要由油酸的氧化产生(Zhao *et al*, 2020)。壬醛则具有鱼腥味、生油脂的味道(刘奇等, 2012)。

表 3 仿刺参性腺酶解过程挥发性成分分析结果

Tab.3 Analysis results of volatile components in *A. japonicus* gonad during enzymatic hydrolysis

化合物 Compound	相对含量 Relative content/%		
	酶解 0 min Hydrolysis for 0 minute	酶解 60 min Hydrolysis for 60 minutes	酶解 120 min Hydrolysis for 120 minutes
2,2,4,6,6-五甲基庚烷 2,2,4,6,6-Pentamethyl-heptane	1.63	1.17	1.27
1,1-二甲基环己烷 1,1-Dimethyl-cyclohexane	–	1.74	1.31
1-甲基-2-羟基降冰片烷 1-Methyl-2-hydroxy-norbornane	5.22	–	–
2,6,11-三甲基十二烷 2,6,11-Trimethyl-dodecane	0.47	–	–
1,1,2-三甲基环戊烷 1,1,2-Trimethyl-cyclopentane	0.01	–	–
1,2-二乙基环丁烷 1,2-Diethyl-cyclobutane	6.17	9.06	–
环癸烷 Cyclodecane	–	0.42	–
1,5-二甲基-6-亚甲基螺环[2.4]庚烷 1,5-Dimethyl-6-methylene- spiro[2.4]heptane	10.84	14.20	13.45
1-甲基-4-亚甲基环己烷 1-Methyl-4-methylene-cyclohexane	–	–	10.54
(Z,Z)-1,3-环辛二烯(Z,Z)-1,3-Cyclooctadiene	0.45	0.59	0.74
2,5,5-三甲基-2-己烯 2,5,5-Trimethyl-2-hexene	0.98	1.13	1.75
6-甲基-1-庚烯 6-Methyl-1-heptene	-	3.73	3.75
(Z)-2-甲基-3-癸烯(Z)-2-Methyl-3-decene	5.25	–	–
(E)-3-十四烯(E)-3-Tetradecene	0.76	–	–
3-庚烯 3-Heptene	–	–	5.16
3-乙烯基环辛烯 3-Ethenyl-cyclooctene	–	–	10.52
环辛烯 Cyclooctene	–	0.55	–
烃类 Hydrocarbons	**31.77**	**32.60**	**48.49**
正己醇 1-Hexanol	–	1.02	1.48
1-辛烯-3-醇 1-Octen-3-ol	2.32	2.01	3.42
(E)-5-癸-1-醇 (E)-5-Decen-1-ol	0.92	1.18	–
乙醇 Ethanol	6.27	5.43	–
1-辛醇 1-Octanol	19.78	31.74	29.35
苯乙醇 Phenylethyl alcohol	–	0.43	–
Z-2-十八烯-1-醇 Z-2-Octadecen-1-ol	–	–	1.70
3-甲基-1-丁醇 3-Methyl-1-butanol	–	2.26	–
庚醇 1-Heptanol	–	–	2.95
醇类 Alcohols	**29.29**	**44.07**	**38.89**
2-十一烷酮 2-Undecanone	0.72	0.70	0.85
4,8-二甲基壬-3,8-二烯-2-酮 4,8-Dimethyl-nona-3,8-dien-2-one	0.26	–	–
3-羟基-2-丁酮 3-Hydroxy-2-butanone	–	1.08	2.19
3-辛酮 3-Octanone	0.38	0.54	–
酮类 Ketones	**1.36**	**2.31**	**3.04**
2-十二烯醛 2-Dodecenal	–	–	0.80
己醛 Hexanal	0.01	–	–
苯乙醛 Benzeneacetaldehyde	–	–	0.56
辛醛 Octanal	3.98	0.94	1.38
壬醛 Nonanal	1.30	0.94	0.68
3-甲基丁醛 3-Methyl-butanal	–	–	0.77
十八醛 Octadecanal	1.24	0.99	–
醛类 Aldehydes	**6.52**	**2.87**	**4.20**

续表

化合物 Compound	相对含量 Relative content /%		
	酶解 0 min Hydrolysis for 0 minute	酶解 60 min Hydrolysis for 60 minutes	酶解 120 min Hydrolysis for 120 minutes
乙酸 Acetic acid	–	5.10	–
壬酸 Nonanoic acid	–	0.77	1.41
6-壬二酸甲酯 6-Nonynoic acid, methyl ester	7.84	11.35	–
对二甲苯 *p*-Xylene	–	–	0.37
2-戊基呋喃 2-Pentyl-furan	0.62	–	1.38
二甲基硫醚 Dimethyl sulfide	22.59	0.94	1.22
其他类 Others	**31.05**	**18.15**	**4.39**

注：“–”表示未检测出。

Note: “–” indicates that it is not detected.

杂环类化合物一般阈值很低，2-戊基呋喃具有类似火腿的香味，对酶解液有一定的增香作用(赵静等, 2016)。二甲基硫醚是海鲜腥味的主要来源之一，酶解前的相对含量高达 22.59%，随着酶解的进行，二甲基硫醚含量显著降低，这与酶解液腥味减弱的现象一致。

3　结论

仿刺参性腺酶解过程中，蛋白质水解成小分子肽及游离氨基酸，一方面使酶解液成分发生了改变，另一方面使其呈现出不同于原液的风味。酶解过程中，可溶性蛋白质含量在前 30 min 内迅速增加，氨基酸态氮含量在 90 min 时达到最高，此时水解度为 43.66%。随着酶解时间延长，酶解液的鲜味和甜味有所增强，这与游离氨基酸中谷氨酸、甘氨酸和丙氨酸含量高的结果一致。电子鼻分析结果表明，仿刺参性腺酶解后气味发生显著变化。通过进一步的 GC-MS 分析发现，酶解后以二甲基硫醚为代表的腥味物质含量显著降低，而一些烃类、醇类、醛类物质的产生赋予了酶解液令人愉悦的气味。

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Changes in the Flavor of *Apostichopus japonicus* Gonads During Enzymatic Hydrolysis

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Abstract　*Apostichopus japonicus* is highly edible and economically valuable. However, because of their self-dissolving characteristics, live sea cucumbers must be processed immediately after being caught. This species produces by-products totaling approximately 30% of the specimen, including sea cucumber intestines, gonads, respiratory trees, and other tissues. At present, the processing and utilization degree of these by-products is still very inadequate, and some are directly discarded, resulting in environmental pollution. According to some studies, sea cucumber gonads are rich in high-quality proteins, which can be used to develop dietary supplement products. Sea cucumber gonads also contain sea cucumber polysaccharides, sea cucumber saponins, active lipids, and other active components. The phenols and flavonoids components in the gonads of Atlantic sea cucumbers are high and a potential source of natural antioxidants. Residents in some parts of the Pacific, such as New Zealand and the Cook Islands, regularly collect gonads from sea cucumber viscera for cooking or as a protein source for traditional meals. In Japan, sea cucumber gonads are processed into expensive functional pill and powder products. However, the development and utilization of sea cucumber gonads in China are still very low. Biological enzymatic hydrolysis is an effective way to improve the utilization of proteins in aquatic products. The enzymatic hydrolysis process degrades macromolecular proteins into small peptides, which are easy to dissolve and

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are conducive to human absorption. At the same time, it is often accompanied by the generation or reaction of peptides, amino acids, and volatile compounds, which in turn causes flavor changes in the enzymatic hydrolysate. The gonads of *A. japonicus* are rich in protein and functional components, with suitable prospects for development and utilization. To explore changes in protein and flavor during the process of enzymatic hydrolysis, neutral protease was used, and the gonads of *A. japonicus* were hydrolyzed using the enzymatic hydrolysis conditions established in previous research. Changes in soluble protein and amino acid nitrogen in the enzymatic hydrolysis process were detected and analyzed, and the flavor changes were studied to provide a reference for the development of related products of *A. japonicus* gonads. The soluble protein content was measured using the Biuret method. The amino acid nitrogen content was measured by the formaldehyde colorimetry method referring to GB/T 5009.39-2003. The hydrolysis degree was determined by Cao´s method, previously established in our laboratory. The composition of free amino acids was analyzed using an automatic amino acid analyzer. The odor characteristics of the enzymatic hydrolysate were analyzed using the PEN3 electronic nose, and the sensory evaluation mechanism was designed to describe the flavor at the same time. The relative contents of volatile components during enzymatic hydrolysis were further analyzed by gas chromatography-mass spectrometry (GC-MS). Results showed that the initial soluble protein and amino acid nitrogen contents in the gonads of *A. japonicas* were 1.14 g/100 g and 0.15 g/100 g, respectively. The soluble protein content increased rapidly within 30 min and then remained constant. The content of amino acid nitrogen gradually increased in the first 90 min and then decreased slightly. The degree of hydrolysis expressed by the changes in soluble protein and amino acid nitrogen during enzymatic hydrolysis reached a maximum at 90 min, up to 43.66%. With the extension of enzymatic hydrolysis time, the umami of enzymatic hydrolysis was enhanced, the fishy smell was weakened, and the sweet and bitter tastes were slightly enhanced. The fresh flavor and sweet taste of enzymatic hydrolysis were also enhanced, consistent with the high glutamate, glycine, and alanine content in free amino acids with the extension of enzymatic hydrolysis time. At the same time, the free amino acid content increased significantly after enzymatic hydrolysis (P<0.05). With the extension of the enzymatic hydrolysis time, the content of free amino acids increased gradually. Glutamic acid is a typical fresh amino acid. TAV(taste activity values) increased from 5.41 to 14.24 at 120 min of enzymatic hydrolysis, significantly contributing to the flavor of the enzymatic hydrolysate. The glutamate content was the highest, followed by glycine and alanine, which also influenced flavor. Moreover, the odor changed significantly during enzymatic hydrolysis. The results of the electronic nose analysis showed that the odor changed significantly after the enzymatic hydrolysis of the gonads of *A. japonicus*. Through further GC-MS analysis, it was found that the content of fishy substances represented by dimethyl sulfide decreased significantly after enzymatic hydrolysis, and the production of some hydrocarbons, alcohols, and aldehydes gave the enzymatic hydrolysis solution a pleasant smell. The types and relative content of hydrocarbons increased significantly, and the content of dimethyl sulfide decreased significantly, likely responsible for the weak fishy odor of the enzymatic hydrolysate. In this study, the changes in soluble protein and amino acid nitrogen during enzymatic hydrolysis were detected and analyzed, and the flavor changes were studied, providing a reference for the development of gonadal-related products of *A. japonicus.*

Key words Sea cucumber gonads; Degree of hydrolysis; Electronic nose; GC-MS; Flavor