

超高压对仙人掌有孢汉逊酵母的损伤机理

雷雨晴, 郝静怡, 吴 傲, 甘芝霖, 孙爱东^{*}

(1. 北京林业大学生物科学与技术学院, 北京 100083; 2. 林业食品加工与安全北京市重点实验室, 北京 100083)

摘 要: 为探究超高压对仙人掌有孢汉逊酵母的损伤机理, 该研究利用流式细胞仪、圆二色谱、扫描电镜与透射电镜等多种方法测定超高压处理(100、200、300、400、500 MPa)对菌体细胞膜、形态结构、胞内物质的影响。试验结果表明: 选择 300 MPa 和 300 s 作为处理条件, 超高压处理后仙人掌有孢汉逊酵母的死亡数为 4.36 lg(CFU/mL), 拥有较好的灭活效果。处理后细胞膜被破坏, 70.50% 的菌体可被碘化丙啶染色, 核酸、蛋白、离子大量外泄, 紫外 260 与 280 nm 吸光度分别达 1.132 及 0.374, 电导率升至 26 μ S/cm, 说明大部分菌体细胞膜通透性提高。同时胞内 Na⁺/K⁺-ATP 酶、Ca²⁺/Mg²⁺-ATP 酶和总 ATP 酶的活力降低, 抑制率分别达到 31.13%, 16.01% 和 20.06%, 核酸、蛋白结构均发生改变, 表明超高压可以影响胞内物质。扫描电镜及透射电镜图像显示, 细胞膜穿孔, 形态结构改变。以上结果表明, 超高压处理对仙人掌有孢汉逊酵母菌体的损伤主要在于破坏细胞膜, 并使得胞内物质失活。研究结果为超高压对非酿酒酵母的损伤机理提供参考。

关键词: 细菌; 杀菌; 仙人掌有孢汉逊酵母; 非酿酒酵母; 超高压

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0 引 言

非酿酒酵母常出现在浆果表面, 并可在果酒天然发酵早期发挥作用, 影响酒类发酵质量, 给产品带来或积极或消极的影响^[1-3]。根据其特性, 可以分为好氧型、低发酵活性型、高发酵活性型三大类, 其中最常见品种为柠檬形克勒克酵母(*Kloeckera apiculata*)、有孢汉逊酵母(*Hanseniaspora uvarum*)两种^[4]。但在果蔬制品(果蔬汁、鲜果、非酒精性饮料等)中, 非酿酒酵母的生长繁殖及代谢会产生乙醇等容易引起品质变的成分, 因而被视为腐败菌种。热处理是杀灭果蔬制品腐败菌的常用手段, 可以有效抑制其生长, 但容易造成热敏性活性物质的损失, 并导致食品感官品质下降^[5]。因此, 以超高压、脉冲电场等为代表的非热杀菌技术开始被应用于食品加工领域, 这些技术可以较好地保持食品原有的品质^[6-9], 同时满足食品卫生要求。

超高压(High Hydrostatic Pressure, HHP)是近年来被广泛研究的一种冷杀菌技术, 在海产品、肉类、果蔬加工等领域均已得到应用^[10-14]。该技术不仅可以有效杀灭病原微生物、延长食品货架期, 还拥有改变食品性质

得到新结构食品的可能性^[14]。相比于传统热杀菌技术, 超高压有效克服了热处理的弊端, 在保证食品的微生物安全的基础上, 还可以较好地保持食品原有的质构、营养、风味以及新鲜度等, 更加符合当前市场对保持食品原汁原味的消费需求^[15]。超高压对食品微生物的杀菌机理一直是近年来的研究热点之一, 目前超高压对于细菌的杀菌研究较为全面和透彻, 已有研究表明, 高压状态下细胞膜损伤、活性氧水平的提升是导致细菌失活的原因^[16]。对于酵母菌, 研究报道超高压对于酿酒酵母的杀菌效果较好, 可以破坏细胞结构使得细胞壁碎片化, 细胞质大量流出^[17], 然而针对非酿酒酵母的杀菌研究却十分少见, 因此本文拟以一种非酿酒酵母-仙人掌有孢汉逊酵母为研究对象探究超高压对其的损伤机理。

仙人掌有孢汉逊酵母是一种较为常见的非酿酒酵母, 可从天然采收的葡萄等浆果中分离出来^[18], 具有生产乙醇的能力并可导致浆果的腐败^[19-21]。浆果采收后, 表面酵母菌的生长繁殖是导致其腐败的主要因素, 因此采取有效措施抑制非酿酒酵母的生理活性对提高产品的质量、减少价值损失具有重要意义。但目前针对杀灭仙人掌有孢汉逊酵母的研究鲜有报道, 因此进行超高压杀菌对其损伤机制的探究是十分必要。

本文中对仙人掌有孢汉逊酵母菌液进行超高压处理, 通过测定碘化丙啶染色情况、紫外 260 及 280 nm 吸光度、电导率等方式表征细胞膜损伤情况, 利用圆二色谱仪、流式细胞仪等仪器探究超高压对菌体胞内物质的影响, 再基于扫描电镜、透射电镜观察得到损伤菌的形

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作者简介: 雷雨晴, 博士生, 研究方向为非热食品加工技术。

Email: reveriecity@163.com

*通信作者: 孙爱东, 教授, 博士生导师, 研究方向为天然产物开发与利用、非热食品加工技术。Email: adsun@bjfu.edu.cn。

态结构变化图像,从试验结果中推断出可能的损伤机理,从而为非酿酒酵母的灭活研究打下基础。

1 材料与方法

1.1 材料与试剂

菌种及培养基:仙人掌有孢汉逊酵母(序列号 FM199954.1),由本实验室从早期腐败的爱国者蓝莓汁中自主分离纯化,采用方法参考本实验室 Zhu 等^[22]的研究内容;麦芽汁琼脂(Malt Extract Agar, MEA)培养基、麦芽汁培养基(Malt Extract Medium, MEM),北京奥博星生物技术有限责任公司。

磷酸缓冲盐溶液(Phosphate Buffered Saline, PBS, pH 值 5.6)、氯化钠、碘化丙啶(Propidium Iodide, PI)、戊二醛、吖啶橙(Acridine Orange, AO),北京拜尔迪生物技术有限公司;超微量 ATP 酶试剂盒(Na^+/K^+ 、 $\text{Ca}^{2+}/\text{Mg}^{2+}$ -T-ATP 酶),南京建成生物工程研究所。

1.2 仪器与设备

CAU-HHP-700-7 型超高压设备,包头科发高压科技有限责任公司;TS-100B 型摇床,上海天呈实验仪器制造有限公司;SW-CJ-2D 型超净台,中兴仪器设备有限公司;LDZX-30KBS 型立式压力蒸汽灭菌器,上海申安医疗器械;TM-03 笔型电导率仪,上海仪电科学仪器股份有限公司;UV 6100 型紫外-可见分光光度计,上海元析仪器有限公司;BD FACS Calibur 型流式细胞仪,上海普迪生物技术有限公司;Quanta FEG250 型扫描电子显微镜,美国 Sprite biotwin 有限公司;Fei tecnai G2 型透射电子显微镜,美国 Sprite biotwin 有限公司;Chirscan plus 型圆二色光谱仪,美国 Photo Physics 仪器公司。

1.3 方法

1.3.1 菌液制备

挑取仙人掌有孢汉逊酵母单菌落接种至 2 mL PBS 缓冲液中,混匀后取 0.5 mL 菌悬液加入 150 mL MEM 培养基,28 °C 摇床 180 r/min 培养 16 h,将培养好的菌液离心(5 000 r/min, 10 min, 4 °C)去上清液,用 PBS 清洗菌体两次,重悬,调整菌液浓度为 $10^6 \sim 10^7$ CFU/mL 备用。

1.3.2 超高压处理

将样品置于 7 L 的超高压密闭圆筒中,以水为传压介质,水温不超过 25 °C,升压速率与降压速率分别为 3.3 MPa/s 和 133.3 MPa/s。恒定压力 500 MPa,处理 150、300、450、600 s;恒定处理时间 300 s,设定压力 100、200、300、400、500 MPa。升降压时间不算在处理时间内。

1.3.3 微生物计数方法

采用 GB 4789.15—2016《食品微生物学检验:霉菌和酵母计数》的方法检测酵母细胞总数。即将菌液用 PBS 缓冲液梯度稀释至合适浓度后取 0.1 mL 通过涂布法接种在 MEA 培养基上,在 (28 ± 0.1) °C 培养箱中培养 48 h 后计数。用酵母菌死亡数 $\lg S$ 表示杀菌效果,公式如下

$$\lg S = \lg(N_0/N)$$

式中 N_0 表示处理前酵母菌数量, N 表示处理后酵母菌数量, CFU/mL。

1.3.4 细胞膜完整性检测

参考 Huang 等^[23]的方法进行细胞膜完整性检测。样品用 PI 染料(终质量浓度为 5 $\mu\text{g/mL}$)在 4 °C 下染色 10 min 后用 PBS 洗涤 2 次备用。用流式细胞仪测定荧光强度,激发和发射波长分别设置为 495 nm 和 615 nm。

1.3.5 细胞膜通透性检测

核酸的共轭双键在紫外 260 nm 处有最大吸光度,蛋白的苯环结构在 280 nm 处有最大吸光度,因此通过光度法测定菌液上清液的 $\text{OD}_{260\text{nm}}$ 及 $\text{OD}_{280\text{nm}}$ 值可以显示核酸与蛋白泄漏情况^[24],即将处理后的菌悬液离心(4 °C, 5 000 r/min, 10 min),取上清液分别在 260 和 280 nm 波长条件下测定吸光度。利用电导率仪测定上清液电导率。

1.3.6 胞内酶活检测

用超微量 ATP 酶试剂盒进行测定,参照试剂盒方法:将处理前后菌液在冰水浴中超声破碎(功率 200 W,工作时间 2 s 间隔 3 s,工作次数 100)得菌体裂解液,稀释至合适浓度后进行酶促反应,与试剂混匀后于 37 °C 反应 10 min,离心(3 500 r/min, 10 min)取上清液,静置 5 min 后在 636 nm 处测定吸光度。胞内酶活力单位以 prot 计,表示为 U/mg。

1.3.7 蛋白质变性检测

参考 Zhong 等^[25]的方法,用圆二色谱仪进行测定。将 1.3.6 中获得的菌体裂解液离心(4 °C, 8 000 r/min, 15 min),取上清液置于圆二色谱仪进行测定,比色皿厚度 0.1 cm,谱带宽度 1.0 nm,分辨率 0.2 nm,扫描波长范围 200~250 nm。

1.3.8 遗传物质变化检测

AO 染料可以对酵母菌细胞中的 DNA 和 RNA 进行染色并测定含量^[26]。将样品用 AO 染液(终质量浓度为 5 $\mu\text{g/mL}$)于 37 °C 避光染色 10 min,用 PBS 冲洗两次洗去多余染料。用流式细胞仪检测其荧光强度,设置激发波长 495 nm,发射波长 615 nm,狭缝宽度 10 nm。

1.3.9 扫描电镜(Scanning Electron Microscope, SEM)与透射电镜(Transmission Electron Microscope, TEM)观察

SEM 与 TEM 样品前处理:将处理前后菌悬液离心(4 °C, 5000 r/min, 10 min)后弃去上清液,菌体用无菌超纯水清洗两次后置于体积分数 2.5 % 戊二醛中,在 4 °C 冰箱中固定 12 h 后用 PBS (pH 值 5.6)漂洗 3 次。进行梯度乙醇脱水,30%、50%、70%、90%乙醇各一次,100%乙醇两次,10 min/次。

对于 SEM 样品,乙醇脱水后用 30%、50%、70%、90%和 100%叔丁醇溶液逐级取代乙醇,真空冷冻干燥后喷金观察。扫描电镜下电子束加速电压 10 kV,放大倍数 5 000 倍。

对于 TEM 样品,乙醇脱水后进行常规渗透,用树脂包埋聚合后进行超薄切片(厚度 70 nm),经染料染色后上镜观察^[27]。

1.4 数据处理

每组试验做 3 次重复,用 Excel 软件处理数据,并使用 SPSS 21.0 软件进行方差分析、Duncan 多重比较及独立样本 t 检验($P < 0.05$),试验结果表示为平均值 \pm 标准差。

2 结果与分析

2.1 超高压处理对仙人掌有孢汉逊酵母的灭活效果

如图 1 所示，当压力在 200 MPa 以下时，几乎无灭活效果，300 MPa 时酵母菌死亡数显著 ($P<0.05$) 提升至 4.36 lg(CFU/mL)，压力继续增大时死亡数缓慢升高，推测 300 MPa 处为高压致死转折点；处理时间小于 300 s 时，已有较好的灭活效果，死亡数达 4.30 lg(CFU/mL)，当处理时间延长时，酵母菌死亡数反而下降，这可能是由于菌体在高压刺激下产生了应激防御^[28]而导致的。考虑到超高压短时处理杀菌不彻底易产生亚致死状态的细胞^[29]（一种存活但非可培养状态，又称“假死”状态），同时 300 s 处为死亡数下降的转折点，因此选定 300 MPa、300 s 作为后续超高压处理的条件。

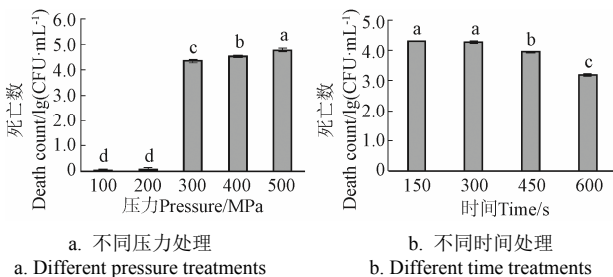


图 1 超高压对仙人掌有孢汉逊酵母死亡数的影响
Fig.1 Effects of HHP on death count of *Hanseniaspora opuntiae*

2.2 超高压处理对细胞膜的影响

2.2.1 超高压处理对细胞膜完整性的影响

PI 是一种可以通过损伤细胞膜与细胞 DNA 结合发出红色荧光的染料，而完整细胞则不会被其染色，常用于细胞活性鉴定^[30]。图 2 结果显示，未处理细胞几乎不被 PI 染色，而超高压处理后则有 70.50% 的细胞被染色，以上结果表明，300 MPa 超高压条件导致了细胞膜损伤。研究表明，细胞膜最重要的功能就是作为选择性屏障保护细胞内部结构，膜完整性受损会导致细胞质流出和膜蛋白失活，进而引发细胞死亡^[31]。

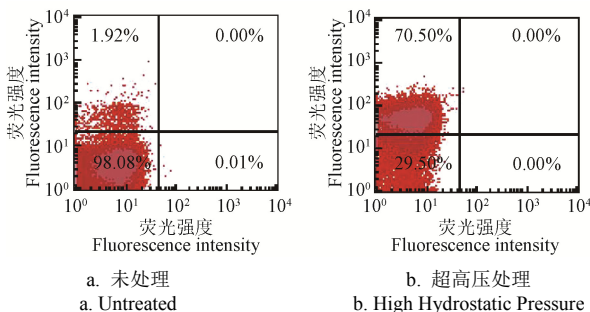


图 2 超高压处理后仙人掌有孢汉逊酵母经碘化丙啶染色的流式细胞图
Fig.2 Fluorescence spectra of *Hanseniaspora opuntiae* stained with PI treated by HHP

2.2.2 超高压处理对细胞膜通透性的影响

当微生物细胞处于不利环境时，会导致细胞内的 K⁺、Na⁺等电解质外泄，从而导致菌悬液电导率提高^[32]。根据表 1 可知，超高压处理后，菌悬液电导率从 4 μS/cm 增加至 26 μS/cm，表明细胞膜通透性升高，细胞内的离子发生外泄，导致电导率发生变化。

蛋白质和核酸作为细胞内重要的大分子物质，在膜通透性正常的情况下不会泄漏到细胞膜外，除非细胞膜通透性发生改变^[33]。通过测定菌液离心后上清液在 260、280 nm 的吸光度可以反映其核酸、蛋白的泄漏情况，而胞内物质的泄漏程度可间接反映膜损伤的情况^[34]。如表 1 所示，超高压处理可导致胞内大分子物质泄漏，OD_{260nm}、OD_{280nm} 值分别提高至 1.132、0.374。以上结果表明，超高压处理酵母细胞膜壁造成了较为严重的破坏，导致细胞膜通透性升高，核酸和蛋白外泄，进而导致菌体死亡。

目前研究学者普遍认为，超高压对微生物的灭活作用是由于其破坏了膜结构，与本文的结果相符。这可能是因为超高压处理可使酵母细胞膜发生相变^[35]，磷脂双分子层上脂肪酰基链聚集，膜流动性降低^[36]，使得自由基积累引发氧化应激反应^[37]，从而造成酵母细胞的失活。

表 1 超高压处理对仙人掌有孢汉逊酵母电导率、紫外 260 与 280 nm 吸光度的影响
Table 1 Effects of HHP on conductivity, OD_{260nm} and OD_{280nm} of *Hanseniaspora opuntiae*

处理 Treatment	电导率 Conductivity/(μS·cm ⁻¹)	紫外 260 nm 吸光度 OD _{260nm}	紫外 280 nm 吸光度 OD _{280nm}
未处理 Untreated	4±1	0.153±0.076	0.038±0.023
超高压处理 HHP	26±1**	1.132±0.098**	0.374±0.039**

注：*表示同一指标不同处理差异显著 ($P<0.05$)，**表示同一指标不同处理差异极显著 ($P<0.01$)。
Note: * indicates the same index had significant differences between different treatments ($P<0.05$), ** indicates the same index had extremely significant differences between different treatments ($P<0.01$).

2.3 超高压处理对细胞形态结构的影响

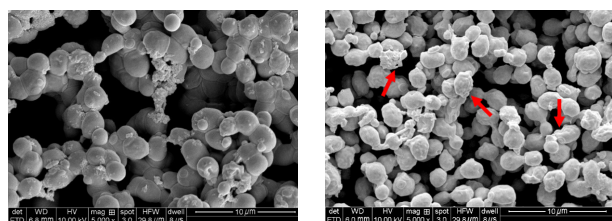
2.3.1 扫描电镜观察

扫描电镜可反映不同处理后细胞外部形态结构的变化。在图 3 中可以发现，未处理的仙人掌有孢汉逊酵母菌呈椭球状，表面光滑完整；在超高压处理后，菌体仍保持椭球状，但表面不再光滑，出现凹陷、裂缝。根据 Shimada 等^[38]的研究，室温条件下，当压力超过 300 MPa 时酿酒酵母的表面形态发生改变，且金属离子开始发生外泄，这与本文的研究结果相似。这表明超高压会导致细胞形态改变，这可能是细胞失活的原因之一。

2.3.2 透射电镜观察

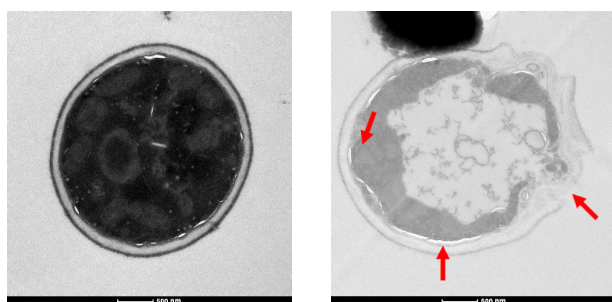
透射电镜可反映不同处理后细胞内部结构的变化。由图 4 可知，未处理的酵母菌体有完整的细胞膜壁，可以看到清晰完整的细胞器，且细胞质均匀致密；经超高压处理后，细胞膜出现明显孔洞，细胞周质空间宽度增大，细胞

器破裂,胞内物质大量外泄。Shimada 等^[38]研究中也发现了类似结果,超高压处理后酿酒酵母内部结构受到影响,大多数细胞器被破坏。Hartmann 等^[39]通过建立酿酒酵母结构模型发现,压力作用在细胞壁上会引起严重的非静水压力,这可能与膜结合蛋白变性等失活机制相关,而非静水压力会被传递到细胞内部并保持稳定,当压力达到 415~460 MPa 时达到临界值,此时可观察到细胞壁损伤,但由于试验用菌种不同,临界值也会存在差异。



a. 未处理
a. Untreated
b. 超高压处理
b. HHP

图 3 超高压处理后仙人掌有孢汉逊酵母的扫描电镜照片
Fig.3 SEM photo of *Hanseniaspora opuntiae* with HHP treatment



a. 未处理
a. Untreated
b. 超高压处理
b. HHP

注: 箭头所指处为被破坏的膜结构及变形的细胞器。
Note: The area indicated by the arrows are the damaged membrane structures and the deformed organelles.

图 4 超高压处理后仙人掌有孢汉逊酵母的透射电镜照片
Fig.4 TEM photo of *Hanseniaspora opuntiae* with HHP treatment

结合扫描电镜和透射电镜图像可以发现,超高压处理会导致膜结构的破坏,增强了细胞膜的通透性,使膜结构穿孔或开裂,从而造成菌体内内容物泄漏,同时超高压处理会导致胞内细胞器结构被破坏,无法进行正常的生理活动,最终导致了酵母的死亡。

2.4 超高压处理对胞内物质的影响

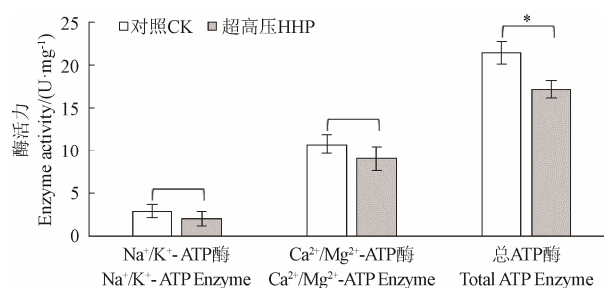
2.4.1 超高压处理对胞内酶活力的影响

Na^+/K^+ -ATP 酶和 $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATP 酶广泛存在于高等真核生物的质膜上,分别负责驱动膜内外 Na^+ 和 K^+ 、 Ca^{2+} 和 Mg^{2+} 离子的对向运动,调节细胞渗透压,从而维持细胞的稳态^[40-41],对信息运送和能量转换等生理活动也具有重要意义^[42]。由图 5 可以看出,超高压处理后,仙人掌有孢汉逊酵母胞内的 Na^+/K^+ -ATP 酶、 $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATP 酶和总 ATP 酶的活力和空白对照相比分别降低了 31.13%, 16.01% 和 20.06% (抑制率)。之前已有研究表明,超高压处理会造成副溶血性弧菌^[43]等菌种的 ATP 酶活降低。本研究结果显示,超高压同样会使得仙人掌有孢汉逊酵母细胞膜 ATP 酶失活,从而导致胞内外离子运输及能量供应失衡,使胞内多种生

理代谢紊乱,导致菌体死亡。

2.4.2 超高压处理对胞内蛋白质结构的影响

圆二色光谱的远紫外区 (200~250nm) 属于蛋白质中肽键的吸收范围,包含着蛋白质主链的构象信息,可以观测蛋白质的二级结构类型。根据圆二色谱结果可知 (图 6),未处理的仙人掌有孢汉逊酵母菌液裂解液在 208 及 222 nm 处显示有明显 α -螺旋的双负峰^[44],但在 216 nm 处没有 β -折叠的特征峰,说明仙人掌有孢汉逊酵母菌胞内蛋白二级结构以 α -螺旋为主。在经过超高压处理后,仍未出现 β -折叠的特征峰,但 208 nm 处峰强度降低,222 nm 处的特征峰消失,说明超高压处理均可以导致 α -螺旋含量下降,对蛋白质二级结构造成影响。研究表明,超高压技术在蛋白改性方面也有广泛应用,导致 α -螺旋和 β -折叠结构含量改变,蛋白结构变得松散^[45]。Gipsy 等^[46]的研究中也发现,超高压会使蛋白二级结构改变。正常细胞内,细胞膜会传递压力,但突破临界值后细胞膜破裂,因此在超高压处理时,细胞膜结构改变后压力作用于细胞器及细胞质,可能是导致内部蛋白结构改变的原因。



注: *表示同一指标不同处理差异显著 ($P < 0.05$)。
Note: * indicates the same index had significant differences among different treatments ($P < 0.05$).

图 5 超高压处理对仙人掌有孢汉逊酵母胞内 ATP 酶活力的影响
Fig.5 Effects of HHP on intracellular adenosine triphosphatase activity of *Hanseniaspora opuntiae*

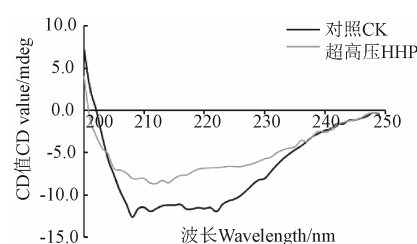
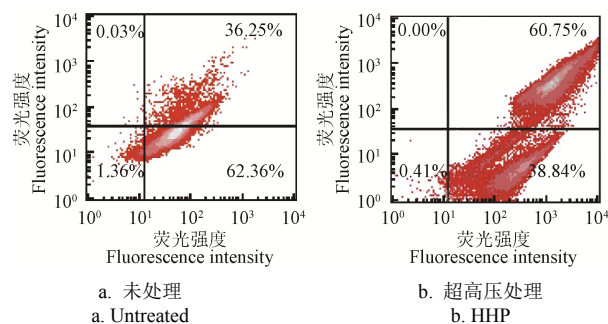


图 6 超高压处理后仙人掌有孢汉逊酵母的圆二色光谱图
Fig.6 Circular Dichroism of *Hanseniaspora opuntiae* with HHP treatment

2.4.3 超高压处理对胞内核酸结构的影响

吡啶橙 (AO) 是一种特异性的荧光染料,具有膜通透性,可以自由透过细胞膜并与菌体细胞内的核酸物质结合^[47]。AO 与双链 DNA 结合,发出绿色荧光;与 RNA 或者单链 DNA 结合,则发出桔红色荧光。根据图 7 结果,超高压处理后,单链核酸增加至 60.75%,而双链核酸降低至 38.84%,表明超高压处理对核酸结构有明显影响。由此可知,核酸损伤是超高压可能的杀菌机制之一。



注：上方象限代表 RNA 或单链 DNA，右下象限代表双链 DNA。
Note: The upper quadrant represents RNA or single-stranded DNA, and the lower right quadrant represents double-stranded DNA.

图7 超高压处理后仙人掌有孢汉逊酵母经吖啶橙染色的流式细胞图

Fig.7 Fluorescence spectra of *Hanseniaspora opuntiae* stained with AO treated by HHP

3 结论

本研究分析了超高压处理对仙人掌有孢汉逊酵母细胞的影响，通过对细胞膜、细胞形态结构、胞内物质变化的研究，探讨了可能的损伤机理，研究结果如下：

1) 超高压处理对仙人掌有孢汉逊酵母有较好的灭活效果，300 MPa 和 300 s 杀灭的菌落数为 $4.36 \lg(\text{CFU/mL})$ 。在对其损伤机理的探究中发现，超高压通过破坏仙人掌有孢汉逊酵母的细胞膜结构使得胞内核酸、蛋白、离子大量外泄，同时，在高压的作用下，胞内 Na^+/K^+ -ATP 酶、 $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATP 酶和总 ATP 酶活均有所降低，胞内蛋白质二级结构改变、DNA 解旋。因此，推测膜的破损、胞内物质的失活是超高压损伤仙人掌有孢汉逊酵母的可能机制。

2) 超高压处理后仙人掌有孢汉逊酵母的生理指标有所改变，但本文研究还不够深入，仅凭所测指标无法阐述损伤具体机制。后续试验可以考虑借助组学研究手段，探究超高压处理后该酵母菌基因层面的变化，从而探究菌体细胞在分子水平上对外界环境做出的应答反应以及相关机制。

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Damage mechanism of *Hanseniaspora opuntiae* by high hydrostatic pressure

Lei Yuqing, Hao Jingyi, Wu Ao, Gan Zhilin, Sun Aidong^{*}

(1. College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, China; 2. Beijing Key Laboratory of Forest Food Processing and Safety, Beijing 100083, China)

Abstract: Non-*Saccharomyces cerevisiae* often appears on the surface of berries in the early natural fermentation of fruit wine, particularly on the most common varieties of *Kloeckera apiculata* and *Hanseniaspora uvarum*. However, the growth, reproduction, and metabolism of the non-*Saccharomyces cerevisiae* can easily produce ethanol and other undesired components, thereby causing the quality changes in most fruit and vegetable products, generally regarded the phenomenon as a spoilage strain. Thermal treatment is a common way to kill spoilage strains in fruit and vegetable juice, fresh fruit and non-alcoholic beverages. This approach can effectively inhibit the growth of spoilage strains. However, the loss of heat-sensitive active substances is accompanied during the treatment, leading inevitably to the decline in sensory quality of food. Alternatively, non-thermal sterilization technology, represented by high hydrostatic pressure (HHP), has widely been applied in food processing, which can effectively overcome the disadvantages of thermal treatment. Most previous studies were relatively comprehensive focuses on the bacteria in the sterilization mechanism of HHP on food microorganisms, but it is still lacking on the non-*Saccharomyces cerevisiae*. Furthermore, the growth and reproduction of non-*Saccharomyces cerevisiae* are the main factors resulting in the microbial spoilage of berry products. Therefore, it is necessary to effectively inhibit the physiological activity of non-*Saccharomyces cerevisiae*, thereby improving the product quality, while reducing the loss of flavor value. One kind of non-*Saccharomyces cerevisiae*, the *Hanseniaspora opuntiae* can be separated from natural berries. Therefore, it is feasible to investigate the damage mechanism of *Hanseniaspora opuntiae* with different sterilization treatments. In this study, *Hanseniaspora opuntiae* was isolated from naturally putrefied Patriot blueberry (*Semen trigonellae*) juice, and then treated with HHP to explore the damage mechanism, where the pressure of 300 MPa and the duration of 300 s were selected as the experimental condition. A flow cytometry, circular dichroism, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were adopted to determine the effects of HHP on the cell membrane, cell morphology and intracellular substances. The experimental results showed that the death count of HHP was 4.36 lg (CFU/mL), indicating an excellent inactivation effect. After treatment with HHP, the cell membrane was destroyed, and 70.50% of *Hanseniaspora opuntiae* was stained by PI. Nucleic acid, protein and ions were leaked out, where the OD_{260nm} and OD_{280nm} reached 1.132 and 0.374, respectively, and the electrical conductivity rose to 26 μ S/cm, indicating that the membrane permeability of most cells increased. Meanwhile, the activities of Na⁺/K⁺-ATPase, Ca²⁺/Mg²⁺-ATPase, and total ATPase decreased, where the inhibition rates reached 31.13%, 16.01%, and 20.06%, respectively. The results of AO staining and circular dichroism spectrum demonstrated that the structure of nucleic acid and protein were changed, proving that the HHP can affect intracellular substances. In the images of SEM and TEM, there were perforations on the cell membrane and outflow of cytoplasm in large quantities. The results indicated that the HHP damage to *Hanseniaspora opuntiae* was due mainly to the destruction of the cell membrane, which caused the intracellular substances to lose their activities in an HHP treatment.

Keywords: bacteria; sterilization; *Hanseniaspora opuntiae*; non-*Saccharomyces cerevisiae*; high hydrostatic pressure