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植物基发酵食品中氰化物的产生与生物控制研究进展

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摘要:在植物基发酵食品的发酵过程中,植物来源的氰苷可进一步转化为氰化物,造成不利影响。因此解析植物基发酵食品中氰化物的产生途径并对其进行控制具有重要意义。氰化物可以从2个方面进行控制:氰化物前体物质的降解和氰化物的酶促降解。作者综述了植物基发酵食品中氰化物的来源及其控制方法,并提出了新的控制策略,旨在为发酵食品中氰化物的控制提供参考。

关键词:氰化物;植物基发酵食品;氰化物降解酶;前体物质

Research Progress in Production and Biocontrol of Cyanide in Plant-Based Fermented Food

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Abstract: During the fermentation of plant-based fermented food, cyanogenic glycosides from plants can be converted into cyanide, which will cause adverse effects. Therefore, it is of great significance to analyze the generation and control strategies of cyanide in plant-based fermented food. The accumulation of cyanide can be controlled by the degradation of cyanide precursors and the enzymatic degradation of cyanide. The author reviews the sources and control strategies of cyanide in plant-based fermented food and proposes new strategies for controlling cyanide in plant-based fermented food, aiming to provide references for the research on cyanide control in fermented food.

Keywords: cyanide; plant-based fermented food; cyanide-degrading enzyme; precursor

植物基发酵食品是发酵食品的重要组成部分,主要以植物为原料,包括发酵谷物、发酵豆类、发酵水果、发酵蔬菜、发酵薯类等。氰苷在植物中广泛存在,在发酵过程中会转化为氰化物。氰化物是一种含CN基团的剧毒物质,成人急性氰化物中毒的参考剂量为20 μg/kg[1]。氰易与细胞色素c氧化酶中的三价铁离子结合,抑制机体正常呼吸作用[2]。中国食品安全国家标准(GB 5009.36—2016)中规定,蒸馏酒及其配制酒中氰化物质量浓度应≤8.0 mg/L。1988年欧洲经济共同体(88/388/EEC)规定,含体积分数40%乙醇的饮料中氰化物最大限量为40 mg/L。研究报道,中国白酒中氰化物质量浓度为0.01~2.29 mg/L[3],葡萄酒中氰化物质量浓度为0.02~0.35 mg/L[4],酸笋中氰化物质量分数为0.80~1.00 mg/kg[5]。

氰化物是氨基甲酸乙酯(ethyl carbamate,EC)的重要前体,氰化物可被氧化成氰酸盐,氰酸盐与乙醇发生化学反应生成EC[2]。EC已于2017年被列为2A类致癌物。因此,有必要对植物基发酵食品中的氰化物进行控制。

作者总结了氰化物及其前体物质的产生途径与控制策略,并综述了新型氰化物降解酶的研究进展,

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旨在为发酵食品中氰化物的控制提供参考。

1 发酵食品原料中氰化物前体物质的形成

氰化物前体物质主要包括蜀黍氰苷、亚麻苦苷和苦杏仁苷等氰苷类物质。氰苷经β-葡萄糖苷酶催化水解产生腈醇，腈醇再通过酶促反应或自发水解释放氰化物[6]。目前已经报道含有氰苷的植物有高粱、大豆、杏仁、樱桃、西番莲科水果、葡萄、竹笋、木薯等[7-8]。

1.1 发酵谷物类食品原料中的氰化物

发酵谷物类食品的主要原料是禾谷科植物，氰化物的前体主要是蜀黍氰苷[9]。在高粱中，蜀黍氰苷主要通过酪氨酸途径生成。酪氨酸经细胞色素P450酶家族中的CYP79A1（EC 1.14.14.36）转化为肟，肟再经细胞色素P450中的CYP71E1（EC 1.14.14.37）催化产生蜀黍氰苷。蜀黍氰苷经β-葡萄糖苷酶（EC 3.2.1.21）水解转化为对羟基苯甲醛后，再经羟基腈裂解酶（EC 4.1.2.11）催化形成氰化物。此外，高粱中N-羟基酪氨酸途径也可形成氰化物[10]。

高粱中总氰化物质量分数为0~33 mg/kg[6]。大麦中亮氨酸来源氰基糖苷质量摩尔浓度约为150 nmol/g[11]。

1.2 发酵豆类食品原料中的氰化物

发酵豆类食品的主要原料是豆科植物，氰化物的前体物质包括来源于缬氨酸的亚麻苦苷和百脉根苷，以及来源于苯丙氨酸的野黑樱苷和蚕豆氰苷[12-13]。亚麻苦苷被β-葡萄糖苷酶水解，产生不稳定的羟基腈中间体——丙酮氰醇。在pH>5或温度高于35 ℃条件下，丙酮氰醇可自然分解为丙酮和氰化物；丙酮氰醇也能被羟基腈裂解酶转化为氰化物[14]。豆科植物中氰化物产生的另一个途径是苯丙氨酸先转化为*R*-扁桃腈，再进一步生成野黑樱苷[15]；而野黑樱苷在β-葡萄糖苷酶的作用下可重新转化为不稳定的*R*-扁桃腈，这一过程伴随氰化物的形成。

有文献报道，棉豆的总氰化物质量分数>2 000 mg/kg[16]，菜豆中含有亚麻苦苷（质量分数为13 479 mg/kg）和百脉根苷（质量分数为33.70 mg/kg）[12]。

1.3 发酵水果类食品原料中的氰化物

发酵水果类食品的主要原料是蔷薇科、桃金娘科等，氰苷由苯丙氨酸途径产生[17]。苦杏仁苷通过β-葡萄糖苷酶水解生成野黑樱苷，随后野黑樱苷转化为扁桃腈；扁桃腈在羟基腈裂解酶的作用下分解为苯甲醛和氰化物。

葡萄中的氰苷主要是（*S*）-野黑樱苷，总氰化物质量分数<10 mg/kg。红葡萄酒中氰化物的质量浓度为0.35 mg/L，白葡萄酒中氰化物的质量浓度为0.02 mg/L，且红葡萄酒比白葡萄酒含有更多葡萄籽释放的氰苷[4]。另外，葡萄皮中还含有少量氰苷[8]。

1.4 发酵蔬菜类食品原料中的氰化物

发酵蔬菜类食品的主要原料是禾本科和十字花科植物等[17]。紫杉氰糖苷是禾本科竹笋中特有的氰苷，可经酶促水解产生氰化物[18]。然而，紫杉氰糖苷的产生途径尚未报道。在新鲜的竹笋中检测到氰苷（质量分数为98.24 mg/kg）和氰化物（质量分数为53~690 μg/kg），发酵后竹笋中氰化物质量分数为0.80~1.00 mg/kg[19-20]。

1.5 发酵木薯类食品原料中的氰化物

发酵木薯类食品的主要原料是大戟科植物。木薯中的亚麻苦苷和百脉根苷是主要的氰化物前体物质[21]。木薯中氰化物质量分数为15~400 mg/kg[22]。

不同植物基发酵食品及其原料种类见表1。植物原料中氰化物的来源相对广泛，涉及粮食作物、蔬菜、豆类、水果。针对发酵食品中有关氰化物的安全性问题，氰化物的控制策略分为2部分：减少氰化物的前体物质，从而间接减少氰化物的形成；转化氰化物并避免形成EC。

2 氰化物前体物质的降解策略

氰苷是氰化物的重要前体物质，氰苷与氰化物的控制可采用“疏通”策略，即促进前体物质转化为氰化物，再降解氰化物。作者以蜀黍氰苷、亚麻苦苷和苦杏仁苷为例来阐述这一策略，其代谢途径见图1。

2.1 蜀黍氰苷的降解策略

Burukutu是一种以高粱为原料发酵生产的乙醇饮料。蒸煮处理可以减少高粱中的氰化物，在沸水处理后，高粱芽苗中的氰化物减少了约75%[50]。Tokpohozin等[51]在高粱糖化过程中添加了产β-葡萄糖苷酶的*Lactobacillus paracasei* ND-34，结果显示高粱汁中蜀黍氰苷减少了69.18%，游离氨基氮增加了22.80%，对羟基苯甲醛增加了11.04%。由

表1　植物基发酵食品及其原料汇总

Table 1　Summary of plant-based fermented food products and their ingredients

发酵食品大类	发酵食品原料	原料分类	原料种类	潜在的氰化物前体物质
发酵谷物类	面包[23]、啤酒（0.01~0.10 μg/L）[24-25]、蒸馏酒（0.01~2.29 mg/L）[26-27]、米酒/清酒[28]、黄酒（0.10~1.03 mg/L）[29-30]、谷物醋[31-32]	禾本科类	高粱*、小米、玉米*、大麦、小麦、大米等	蜀黍氰苷[33]
发酵豆类	酱油[34]、豆豉[35]、豆腐[35]、豆酱[36]、发酵大豆[37]、发酵豆浆[38]	豆科	大豆*、蚕豆*、四季豆	亚麻苦苷、百脉根苷[12]、野黑樱苷和蚕豆氰苷[13]
发酵水果类	果醋[39]、葡萄酒（0.02~0.35 mg/L）[40-41]、发酵水果汁[42]、发酵水果[43]等	蔷薇科、桃金娘科、芭蕉科等	葡萄*、苹果*、梨、菠萝、番石榴*、樱桃*、李子*、杏仁*、百香果、香蕉等	苦杏仁苷和野黑樱苷[17]
发酵蔬菜类	酸菜[27,44]、泡菜[34]、腌菜[45]、发酵黄瓜[46]、酸笋（0.80~1.00 mg/kg）[5]等	十字花科、木樨科、伞形科、葫芦科、禾本科等	卷心菜、橄榄、胡萝卜、黄瓜、刺山柑、竹笋*等	紫杉氰糖苷[18]
发酵木薯类	Gari（20.5~25.4 mg/kg）[47]、Fufu（20.0~24.3 mg/kg）[48]、Peujeum[49]、Agbelima（22.5~49.0 mg/kg）[48]等	大戟科	木薯*	亚麻苦苷和百脉根苷[21]

注：*表示该植物中含有氰化物前体物质。

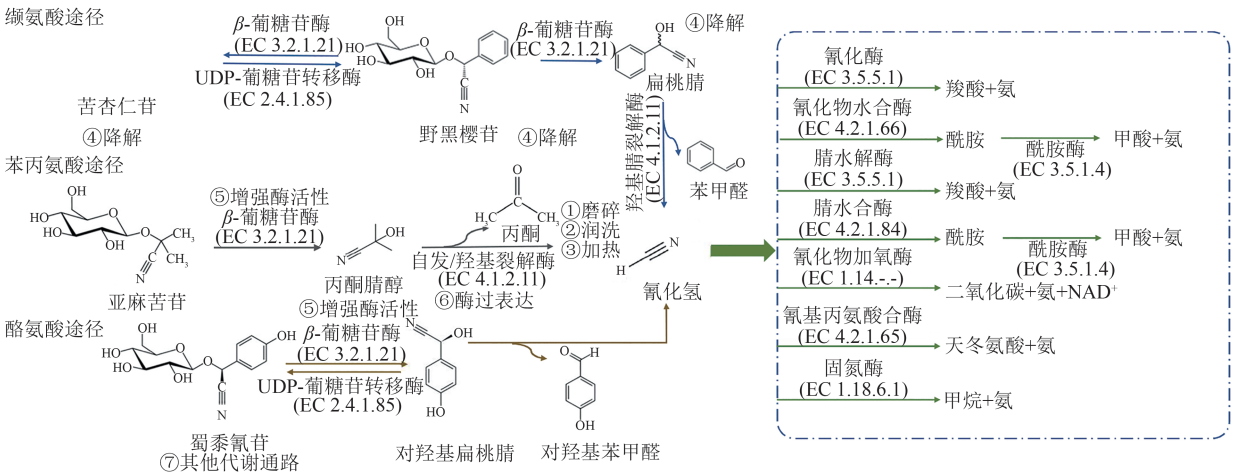


图1　三种氨基酸来源氰化物的代谢途径

Fig. 1　Metabolic pathways of cyanide from three amino acid sources

此可知，β-葡萄糖苷酶推动了“蜀黍氰苷—氰化物—氨”这一途径的物质转化，说明“先疏通后降解”的策略是可行的。Bjarnholt等[52]用谷胱甘肽转移酶（EC 2.5.1.18）构建了蜀黍氰苷转化生成对葡糖氧基苯乙酸的新途径，避免了氰化物的产生。

2.2　亚麻苦苷的降解策略

Zhong等[53]发现超声处理木薯汁可以促进β-葡萄糖苷酶降解氰苷果，氰苷和氰化物分别减少了24.96%和40.36%。Obilie等[54]研究了木薯发酵食品Akyeke的加工过程，发现磨碎的木薯经过*Lactobacillus*发酵后，可有效降解氰苷（降解率>99%）。Narayanan等[55]通过过表达羟基腈裂解酶显著降低了植株中丙酮氰醇和氰化物的含量。由于氰苷和腈醇之间可以互相转化，因此丙酮氰醇的减少促进了亚麻苦苷向丙酮腈醇方向的转化。

2.3　苦杏仁苷的降解策略

苦杏仁苷的控制可以通过降解扁桃腈来实现。腈水解酶将扁桃腈转化为扁桃酸，从而避免形成苯甲醛和氰化物[56]。

当氰化物被氰化物降解酶转化为甲酸后，由于氰苷和腈醇可以自发形成氰化物，因此无法确定氰化物是否减少。氰化物的控制策略应同时考虑两点，一是降解氰苷与腈醇，二是降解氰化物。因此，氰化物的控制涉及氰化物及其前体物质。采用破碎、蒸煮、菌株发酵等多种手段相结合的方式，可实现对氰化物的高效控制。

3 氰化物的酶促降解策略

目前，氰化物的酶促降解研究较多，已有研究证实腈水解酶、氰化物水合酶（EC 4.2.1.66）和氰化酶（氰化物二水合酶）（EC 4.2.1.104）等具有降解氰化物的活性。根据序列相似性，腈水解酶、氰化酶和氰化物水合酶均属于腈水解酶超家族[57]。用于发酵食品中降解氰化物的酶主要是腈水解酶超家族成员。其中，腈水解酶能够催化腈类物质水解生成相应的酸；而氰化酶和氰化物水合酶优先以氰化物为底物，并分别产生羧酸和酰胺。然而，在发酵食品中利用各种氰化物降解酶实现氰化物的有效控制仍面临诸多挑战。

目前，氰化物的酶促降解策略包括水解、水合、氧化、硫氰酸盐降解、取代以及还原反应等多种机制。不同降解酶的来源及产物见表2。

表2 氰化物降解酶的来源及产物

Table 2 Sources and products of cyanide-degrading enzymes

降解酶	来源	降解底物	产物	参考文献
氰化物水合酶	*Fusarium oxysporum* N-10	金属氰化物	甲酰胺	[58]
	Fusarium solani	氰化钾或金属络合氰化物	甲酰胺	[59]
	Neurospora crassa	氰化钾	甲酰胺	[60]
	Aspergillus nidulans	氰化钾	甲酰胺	[60]
	Gloeocercospora sorghi	氰化钾	甲酰胺	[60]
	Gibberella zeae	氰化钾	甲酰胺	[60]
	Fusarium solani	氰化物与金属络合氰化物	甲酰胺	[61]
	Leptosphaeria maculans	氰化钾	甲酰胺	[62]
	Aspergillus niger K10	氰化氢	甲酰胺	[63]
腈水解酶	*Pseudomonas fluorescens* NCIMB11764	肉桂腈	肉桂酸和氨	[64]
	Thermophilic bacillus Dac521	4-氰基吡啶和苯甲腈		[65]
	Pseudomonas fluorescens DSM7155	2-（甲氧基）-扁桃腈	羧酸和氨	[66]
	Labrenzia aggregata	邻氯扁桃腈	邻氯扁桃酸和氨	[67]
氰化酶	*Pseudomonas stutzeri* AK61	氰化物	甲酸盐和氨	[68]
	Bacillus safensis PER-URP-08	氰化物	氨和二氧化碳	[69]
	Saccharomyces cerevisiae	氰化物	甲酸	[70]
	Bacillus pumilus C1	氰化钠	氨	[69]
腈水合酶	*Rhodococcus erythropolis* N771	丁基异腈	叔丁胺	[71]
	Pseudomonas putida NRRL-18668	2（*S*）-（4-氯苯基）-3-甲基丁腈	2（*S*）-（4-氯苯基）-3-甲基丁酰胺	[72]
	Rhodococcus erythropolis A4	苯甲腈	苯甲酰胺	[73]
氰化物加氧酶	*Klebsiella pneumoniae*	硫氰酸盐		[74]
	Ralstonia sp.	硫氰酸盐		[74]
	Pseudomonas fluorescens NCIMB11764	氰化物	含氮生长底物	[75]
硫代硫酸氰硫转移酶	*Klebsiella pneumoniae*	硫氰酸盐		[74]
	Ralstonia sp.	硫氰酸盐		[74]
	Thiobacillus thioparus THI112	硫氰酸盐	氨和羰基硫	[76]
	Thiobacillus thioparus THI115	硫氰酸盐	氨和羰基硫	[76]
β-氰基丙氨酸腈酶	拟南芥	*β*-氰基-丙氨酸	天冬氨酸	[77]
	Variovorax boronicumulans CGMCC4969	*β*-氰基-丙氨酸	氨	[78]
固氮菌	*Azotobacter vinelandii*	金属氰化物	氨气和甲烷	[79]
	Klebsiella oxytoca	氰化物	甲烷和氨	[79]
酰胺酶	*Fusarium oxysporum* N-10	甲酸酯	氨	[58]
	Fusarium solani	甲酸酯	氨	[61]
	大麦	*β*-氰基丙氨酸	氮源	[80]
	Klebsiella oxytoca	氰化物		[81]

3.1 水解反应与水合反应

氰化物水合酶主要来源于真菌。氰化物或金属络合氰化物等底物可诱导氰化物水合酶的转录[82]。真菌可以利用氰化物或甲酰胺作为氮源生长[83]。氰化酶则来源于细菌。氰化物水合酶催化氰化物生成甲酰胺，氰化酶催化氰化物生成甲酸和氨。甲酰胺酶（EC 3.5.1.49）能够催化甲酰胺分解为氨和甲酸，而甲酸在甲酸脱氢酶（EC 1.2.2.1）的作用下进一步转化为二氧化碳。

腈水解酶将腈降解为羧酸。腈水解酶根据不同的底物特异性分为脂肪腈酶（EC 3.5.5.7）、芳香乙腈酶（EC 3.5.5.5）、芳基乙酰腈酶（EC 3.5.5.1）。Shen 等[70]将含有腈水解酶的 *Saccharomyces cerevisiae* MT-1S 首次应用于白酒发酵体系来降解氰化物，成功降解了 46.45% 的氰化物。*S. cerevisiae* MT-1 分离自白酒体系，这种原位体系来源的功能微生物的应用策略有效避免了外来微生物引起的食品安全问题。通过固定化技术可以保留腈水解酶的酶活性，提高其重复利用率[84]。

腈水合酶催化腈的水合反应形成酰胺，该酶由 α 和 β 两个亚基组成。腈水合酶通常与酰胺酶一起构成腈水合酶-酰胺酶系统，最终腈被转化为羧酸和氨。

3.2 氧化反应

氰化物加氧酶（EC 1.14.-.-）包括氰化物单加氧酶与氰化物双加氧酶[75]。氰化物单加氧酶将氰化物转化为氰酸盐，随后氰化酶催化氰酸盐分解为氨和二氧化碳。而氰化物双加氧酶则能直接将氰化物转化为氨和二氧化碳。

3.3 硫氰酸盐降解反应

硫代硫酸盐氰化物硫转移酶（EC 2.8.1.1）对硫氰酸盐的降解主要有 2 条途径，且均在碱性条件下进行。第一条途径是将硫氰酸盐转换成氰酸盐，再由氰化酶将氰酸盐转化为氨和二氧化碳。第二条途径则是直接将硫氰酸盐转化为氨和羰基硫[76]。

3.4 取代反应

β-氰基丙氨酸腈酶（EC：3.5.5.4）催化 β-氰基丙氨酸生成天冬氨酸和氨。β-氰基丙氨酸腈酶是植物中氰化物降解途径的最后一步[78]。

3.5 还原反应

氰化物在固氮酶（EC 1.18.6.1）的催化下分解为氨和甲烷。从工业废水中分离出的 *Klebsiella oxytoca* 能够将氰化物降解为氨和甲烷[79]。外源性氨和亚硝酸盐会抑制固氮酶产生菌对氰化物的降解作用[79]。

4 氰化物的基因组学控制策略

腈水解酶产生菌是降解氰化物的常用功能微生物。除了常规的培养筛选，鉴于腈水解酶基因序列具有高度同源性这一特点，有研究者通过探针或组学等技术来挖掘新的氰化物降解酶产生菌。

在发酵食品体系来源的宏基因组学数据中，可利用目标酶基因序列设计特异性引物扩增方法，筛选潜在的含腈水解酶基因的菌株[85]。Shen 等[70]采用宏转录组测序挖掘出与腈水解酶高度同源的转录活性基因序列，进而筛选出一株可降解氰化物的微生物——*S. cerevisiae* MT-1[70]。

从现有的腈水解酶超家族数据库中查找目标酶基因序列，对目标序列进行分子改造或异源表达[86-87]。目前，表达数据库中的基因所需技术已十分成熟[88]。Veselá 等[89]挖掘 GenBank 数据库建立腈水解酶超家族数据集，根据其底物特异性将数据集分为芳香腈酶、芳基乙腈酶、脂肪族腈酶与氰化水合酶共 4 种亚型。针对特定底物搜索相应腈水解酶基因序列，可挖掘新的氰化物降解酶。

5 展望

植物基发酵食品中氰化物的控制应予以重视并寻求有效解决方案。目前，氰化物控制策略可分为 2 个方面：一是减少氰化物的积累；二是促进氰化物的降解并避免形成乙腈。迄今为止，已表征的氰化物降解酶基因序列十分有限，不利于新的氰化物降解酶与降解菌的挖掘。传统的可培养筛选方法仍是扩充氰化物降解酶数据库的有效方法。通过宏基因组文库有望挖掘高性能的氰化物降解酶。目前，关于氰化物降解酶基因序列的表征研究较少，因此需要进一步开发准确高效的高通量鉴定方法。

此外，氰化物降解酶的催化机制、调控机制以及降解酶产生菌对实际发酵体系的影响等方面有待深入研究，以有效评估其应用效果。氰化物的控制涉及氰化物代谢途径上下游的多种物质。功能菌株的选育及高效能综合策略的探索，有助于实现植物基发酵食品中氰化物的有效控制。

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