脉冲电场技术通过氧化应激途径诱导营养物质合成和杀灭微生物的研究进展

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摘要：脉冲电场（Pulsed Electric Field，PEF）技术是一种新兴非热食品加工技术，由于PEF对食品质量损伤小且能杀灭食品中有害微生物所以被广泛应用。PEF通过氧化应激途径激活植物类食品的营养物质合成途径，诱导营养物质合成、提高食品质量，然而关于这一领域的研究较少。PEF 发生了氧化应激反应造成了营养物质合成与积累，植物类食品受到 PEF 刺激后产生大量活性氧（Reactive Oxygen Species，ROS），ROS会激活代谢物合成途径最终合成蛋白、多酚、硫代葡萄糖苷和胡萝卜素等物质；ROS聚集在微生物的细胞膜上会造成蛋白表达异常，损害脂质层和脱氧核糖核酸（Deoxyribonucleic Acid，DNA），最终导致微生物失活。

关键词：脉冲电场技术；活性成分；氧化应激；抗氧化；生物活性；杀菌

文章编号：1673-9078(2023)12-345-353 DOI: 10.13982/j.mfst.1673-9078.2023.12.1343

Research Progress on the Nutrient Synthesis and Microbial Inactivation Induced by Pulsed Electric Field Technology via the Oxidative Stress Pathway

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Abstract: PEF technology is an emerging non-thermal food processing technology, and has been widely used because of its little damage to food quality and ability to kill harmful microorganisms in food. PEF activates the nutrient synthesis pathway of plant foods through the oxidative stress pathway, induces nutrient synthesis, and improves food quality. However, there are few studies in this field. PEF intiates oxidative stress reactions, resulting in the synthesis and accumulation of nutrients. Plant foods are stimulated by PEF to produce large amounts of ROS, which activate metabolite synthesis pathways, eventually leading to the synthesis ofproteins, polyphenols, thioglucosides and carotenoids; The accumulation of ROS on the cell membranes of microorganisms would cause abnormal protein expression, damage to the lipid layer and DNA, and eventually cause microbial inactivation.

引文格式：

刘思敏,王谦鑫宏,常钧棋,等.脉冲电场技术通过氧化应激途径诱导营养物质合成和杀灭微生物的研究进展[J].现代食品科技,2023,39(12):345-353

LIU Simin, WANG Qianxinhong, CHANG Junqi, et al. Research progress on the nutrient synthesis and microbial inactivation induced by pulsed electric field technology via the oxidative stress pathway [J]. Modern Food Science and Technology, 2023, 39(12): 345-353

收稿日期：2022-10-23

基金项目：吉林省科技计划项目（20200404066YY）

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Key words: pulsed electric field technology; active ingredient; oxidative stress; antioxidant; bioactive; sterilization

传统的食品加工技术会存在对食品的营养价值和食品安全产生不利影响的隐患，可能会造成营养流失，有害物质增加，最终影响人体健康[1,2]。传统食品加工技术主要应用在营养物质提取，微生物及其孢子的灭活，活性酶的灭活等方面，多数技术已经形成大规模工业化生产，比如热处理技术[1,3,4]。工业化生产的代价就是牺牲食品本身新鲜口感提高食品生产效率[5,6]。此外，许多营养物质具有热敏性，在热处理时会因热降解而失去生物活性，不利于营养物质的积累[7]。

传统食品加工技术给食品带来的营养流失、试剂残留和化学添加剂过多等一系列问题开始让人们对食品安全产生担忧[8]。所以人们迫切寻找一种新兴食品加工技术，这些技术不仅减少了营养物质流失，增加食品安全性，甚至可以通过刺激食品自身的生物合成的代谢途径来生成人们所需要的一些重要化合物，例如酚类、胡萝卜素和蛋白等，这些新兴技术通过生物合成途径来改善食品的质量[9-11]。这些技术中包括高静压、脉冲电场、超声，紫外线等[12-17]。

由于近年来PEF在食品加工应用中的优势越来越明显，所以关于PEF技术在食品中应用的研究越来越多[18]，PEF相对于其他技术的优势是能降低对食品中营养物质的损害，延长保存时间，杀灭食品中有害微生物[19-23]。PEF是一种脉冲发射持续时间为微秒至毫秒、强度为0.1~80 kV/cm的强电场短脉冲技术[23]，现在主要研究方向是在食品的有效物质提取、微生物灭活、干燥和解冻等领域[24-26]，但是关于脉冲电场技术通过影响生物代谢途径来诱导营养物质合成的研究较少。

植物受到PEF刺激后会分泌大量代谢产物来保护机体免受氧化应激损伤，增加营养物质的积累[27-29]。PEF处理后的植物组织中蛋白和多酚类等这种初级和次级代谢产物含量会大幅增加。这些植物类食品受到PEF处理后产生的抗氧化的代谢产物正是保护人体细胞免受氧化应激损伤的营养成分[30,31]。

植物细胞为了应对外界刺激发生即时反应，释放出胁迫信号因子激活信号传导网络，继而参与转录因子的合成，最终生成代谢产物，这是植物食品在受到PEF处理后的应激生理学[32-35]，营养物质因此而增加[36]。PEF增加了细胞膜的通透性，这一特征触发了细胞的应激反应，类似于伤害应激[37]，然后分泌大量抗氧化物质保护机体免受伤害。此外，过量的ROS积累导致氧化应激也会导致微生物死亡，达到杀菌的目的[38-40]。

本文综述了PEF通过氧化应激途径诱导植物营养物质的合成提高有效成分提取率。此外还总结了PEF通过氧化应激途径杀灭微生物的机制，为PEF技术通过氧化应激途径影响食品质量的研究方向提供参考。

1　PEF 诱导植物性食品营养物质合成

早期研究发现PEF通过刺激细胞从而使细胞内的活性氧（ROS）的产生量大量增加[41]。ROS的产生与细胞的健康状态紧密相关，当细胞遭到外界刺激损伤，植物细胞的一个共同的特征应激反应就是产生ROS。当细胞膜受损破裂后，ATP从受损的细胞中释放出来，与细胞膜受体结合后产生ROS[42,43]，此过程还会产生乙烯与茉莉酸，所有这些次生胁迫信号分子都会通过细胞扩散，并启动信号转导网络，导致转录因子的激活，从而植物体内的营养物质合成[44]，产生出的ROS和乙烯会诱导酚类化合物的生物合成[45]，而乙烯和茉莉酸会诱导硫代葡萄糖苷的生物合成[46]。另一方面，ROS会诱导类胡萝卜素类化合物合成[47]。

1.1　酚类和胡萝卜素

Galind等[48]在马铃薯组织上施加PEF，使组织产生创伤，继而诱导一系列代谢发生。首先大量ROS爆发式产生[49]，与受体结合后产生大量的初级和次级代谢产物。马铃薯组织恢复阶段时，将产生大量绿原酸来提高组织对氧化应激的保护[50]，接下来将合成多肽来增加蛋白质的含量应对氧化应激损伤。这一研究发现为我们通过PEF刺激植物组织来提高代谢产物产量提供研究思路，如图1所示。

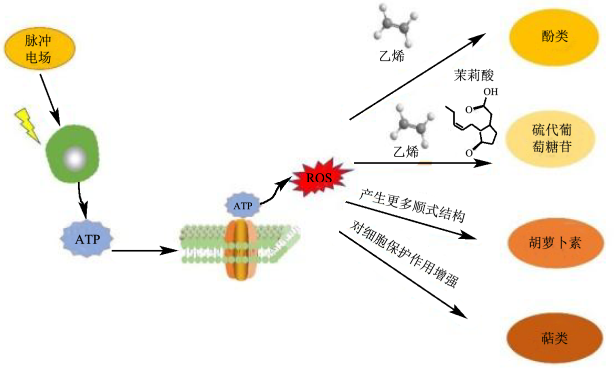

图1　PEF通过氧化应激途径诱导食品中营养物质积累

Fig.1 PEF induces nutrient accumulation through the oxidative stress pathway

Leong等[51]研究了PEF处理不同种类胡萝卜，通过体外实验考察这些提取物对过氧化氢氧化损伤后的细胞的保护作用。结果发现，0.8 kV/cm条件下得到的Yellow Solar Purée提取物可显著增加Caco-2细胞的活力，并抑制乳酸脱氢酶（Lactate Dehydrogenase，LDH）

泄漏和 NO 的产生。PEF 提取的胡萝卜提取物对细胞活力起到很好的保护作用，能够使氧化应激（含 H_2O_2）细胞的活细胞数和 NO 产生百分率恢复到与非应激（无 H_2O_2）细胞相似的水平。此外还发现，PEF 提取物对细胞保护能力与总胡萝卜素含量与总花青素含量呈线性关系。对于总胡萝卜素含量较低的 Yellow Solar，PEF 能显著提高它的胡萝卜素含量[51,52]。Leong 等[53]发现 PEF 处理后可以缩短对 VitisVinifera 的浸泡时间并提高对 H_2O_2 氧化胁迫的生物保护作用。Leong 等[54]又评价了脉冲电场对黑比诺葡萄汁花色苷释放及保健作用的影响，同样得到 PEF 处理后的黑比诺葡萄能够生成具有更强抗氧化活性的物质，并表现出更强的保护细胞免受氧化应激的能力。

PEF 加工后的番茄含有的叶黄素、胡萝卜素、反式番茄红素、顺式番茄红素的浓度都远高于热处理加工的含量，这是因为番茄受到 PEF 的伤害后，受损细胞释放出 ATP，ATP 作为创伤信号在细胞空隙间扩散，激发了番茄的应激反应，增强了番茄的代谢活性并合成大量营养物质[55-58]。应用 PEF 处理可生产出更多的高水平顺式异构体的营养素，顺式异构体的营养物质在人体内的生物利用率更高[36]，如图 1 所示。Martínez 等[59]采用 PEF 技术辅助提取 Dried Rhodotorulaglutinis Yeast 中类胡萝卜素，通过对实验条件的优化提高 PEF 对类胡萝卜素的提取率，未使用 PEF 处理的提取率为 20%，使用 PEF 处理后提取率增加到 80%。Kokkali 等[60]研究发现 PEF 处理后的微藻生物提取物的抗氧化能力显著增强。通过对实验因素考察发现提取溶剂类型和提取时间能显著影响微藻生物中类胡萝卜素和酚类化合物的提取效率，这表明溶剂和时间会影响 PEF 对植物体内营养物质的积累。PEF 能提高营养物质在植物类食品内合成的数量，可以利用这项优势为含量低的活性物质定制专属提取参数，利用 PEF 技术合成更多人们所需要的营养物质[51,52]。

1.2 皂苷类

皂苷类成分对身人类身体健康有很大的益处，如促进抗体的产生和保护神经、抗抑郁、提高免疫力、抗焦虑、保护心脏、抗炎、抗细菌、抗哮喘和止泻作用等[61-65]。

Liu 等[66]研究了脉冲电场提取的人参皂苷对过氧化氢诱导的 HEK-293 细胞氧化应激的保护作用，实验结果发现PEF提取物对细胞的保护作用强于溶剂冷浸法提取物。加入 PEF 提取物后显著降低了 HEK-293 细胞活性氧的积累，使 HEK-293 细胞保持了更健康的细胞状态。说明 PEF 提取的人参总皂苷具有更强的细胞抗氧化能力。

Hou 等[67]对脉冲电场的电场强度和频率进行优化，在电场强度为 20 kV/cm、频率为 6 000 Hz、φ=70%乙醇-水溶液、速度为 150 L/h 的条件下，人参皂苷得率最高，为 12.69 mg/g。与微波辅助提取法、热回流提取法、超声波辅助提取法、溶剂提取法和超高压提取法等方法进行比较，PEF 的人参皂苷提取率明显增高，而且时间缩短。Lu 等[68]为了增加人参皂苷的产率，采用了 PEF 结合 β-葡萄糖苷酶法提取人参总皂苷。在单极脉冲模式下，PEF 对 β-葡萄糖苷酶的活性没有影响[69]。根据响应面法得出电场强度 15 kV/cm，脉冲次数 10 次，酶质量分数 2%的最优条件，在该条件下人参总皂苷的提取率为 38.15 mg/g，明显加快了提取时间，增大了提取效率。脉冲电场技术提取皂苷类成分，不仅加大了提取效率，更是使皂苷类的生物活性能力显著提高，这为我们以后提取具有更高生物活性类的皂苷类成分提供了新的研究方向。

1.3 硫代葡萄糖苷

硫代葡萄糖苷由于具有抗菌、保护神经和抗癌等作用，所以在制药和食品工业中具有特殊的地位[70-75]，但是具有水溶性和热敏性的特征，所以它们在加热过程中会有很大的损失，提取这类成分时候尽量避免热处理[74,75]。硫代葡萄糖苷在植物组织的亚细胞间隔中保持稳定化学性能和生物活性，当细胞破裂后黑芥子酶会与硫代葡萄糖苷接触，将硫代葡萄糖苷水解，所以硫代葡萄糖苷的损失与黑芥子酶的是否灭活紧密相关，PEF 能否通过对黑芥子酶灭活来达到保留硫代葡萄糖苷的目的也成为新的研究对象[76-78]。

西兰花中的黑芥子酶催化硫代葡萄糖苷酸使其发生酶解反应，生成其他生物活性产物，这些产物的含量被认为是影响西兰花产品质量的主要因素。Frandsen 等[79]发现 35 kV/cm 的 PEF 处理会使黑芥子酶失去活性，但同时发现大多数硫代葡萄糖苷在 PEF 处理之前已被降解，如果保证 PEF 能成功提取硫代葡萄糖苷需要通过高压处理使黑芥子酶失活。

Ingrid 等[80]采用脉冲电场技术对西兰花花茎中硫代葡萄糖苷进行提取。实验结果发现 PEF 预处理后的西兰花花茎中葡糖豆素、硫萝卜素、芥菜素和新芥菜素增加了大约两倍，同时还发现黑芥子酶仍然保持活性。这个实验发现 PEF 可以在酶没有失活的条件下，加大硫代葡萄糖苷类化合物的提取效率，这可能成为新的研究方向。

表 1 PEF 通过氧化应激途径对生物活性物质合成的影响

Table 1 Effect of PEF on the synthesis of bioactive substances through the oxidative stress pathway

项目	材料	诱导途径	结果
酚类和胡萝卜素	马铃薯	氧化应激	产生大量绿原酸和蛋白质
	胡萝卜	氧化应激	类胡萝卜素和和花青素的含量显著增加
	黑皮诺葡萄	氧化应激	有效化合物含量显著增加，提取液表现出对细胞更好的保护作用
	西红柿	氧化应激	产生更多的顺式异构体的化学成分，这类化合物能更好的被人体吸收利用
萜类	人参	氧化应激	提取出的人参皂苷能使细胞形态更加完整
硫代葡萄糖苷	西蓝花	氧化应激	使西蓝花中硫代葡萄糖苷类成分含量显著增加

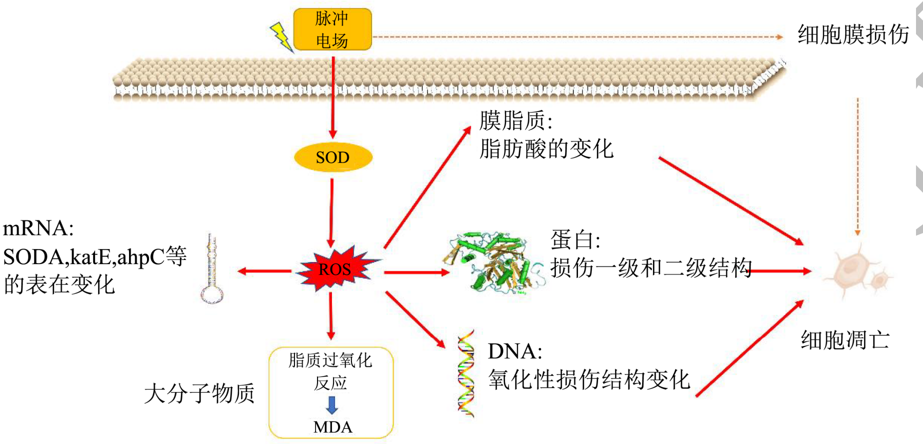

图 2 PEF 通过氧化应激途径诱导细菌凋亡

Fig.2 PEF induces bacterial apoptosis via oxidative damage pathway

Naima 等[81]对完整的黑墨西哥甜玉米细胞施加了脉冲电场，发生的电渗透诱导了一代氧物种（氧化跳跃），这种氧化跳跃与电渗透呈线性关系，但是与细胞活性丧失没有相关性，这样的结果与哺乳动物细胞观察到的结果不同，表明即使在对哺乳动物或完整的植物细胞进行脉冲处理时观察到相同的现象，相关的代谢反应也不同。

2　PEF 通过氧化应激途径诱导微生物凋亡

PEF 是一种常温条件下使食品中微生物灭活的新技术。PEF 在食品灭菌中的应用不仅不会改变食品的新鲜度和口感，而且能保证灭菌的质量。PEF 对微生物的灭活的原理是由于微生物的细胞膜在 PEF 的的作用下发生不可逆穿孔孔并使半渗透屏障遭到破坏[82]。除此以外，PEF 诱导的氧化应激反应会改变细菌的代谢，从而诱导微生物的凋亡。微生物的灭活意味着能保留更多食品中的营养物质和降低口感的损失[82,83]。

孟媛媛等[84]从 ROS 介导的氧化应激角度探讨 PEF 对金黄色葡萄球菌的作用机制。经 PEF 处理后，金黄色葡萄球菌内的 ROS 水平升高，过量的 ROS 诱导严重的氧化应激反应，导致脂质过氧化、DNA 氧化和细胞膜脂肪酸损伤、蛋白质和 DNA 的结构损伤以及氧化应激相关基因的表达发生显著异常，最终导致 ROS 介导的细菌细胞凋亡[84]，如图 2 所示。

Liu 等[85]以质谱为基础比较了 PEF 处理前后大肠杆菌蛋白表达变化。已有文献表明活性氧（ROS）的过度积累可以显著降低超氧化物歧化酶缺乏的肠沙门氏菌中 AcnB 的蛋白水平[86]。另一种上调的酶是双功能脯氨酸利用酶 A（PutA；A0A0G3K113），PutA 在大肠杆菌中的缺失已被证明会增加细胞对氧化损伤的敏感性[87]。对大肠杆菌施加 PEF 后膜透性增加，这不仅增加有害物质进入细胞，还会使细胞产生渗透失衡[88]。足够强的电场能够在细胞膜上造成不可修复的穿孔，导致不可逆的细胞损伤、破坏，并最终死亡[89]，如图 2 所示。一般认为当电场强度达到 5~15 kV/cm 时，细胞会发生不可逆损伤。这与观察结果一致，即 15 kV/cm 以上强度的脉冲电场对膜击穿是必要的[90,91]。为了保证微生物的灭活率，所以要保证场强为 25 kV/cm 或以上的电场电场来确保食品加工中病原体的灭活[92]。

3　结论

PEF 作为一种非热处理的食品加工技术，通过氧化应激途径来增加营养物质和杀灭微生物是一个新的研究方向。在食品加工领域中能将营养物质合成并累积到最大量正是食品加工工艺里最稀缺的。PEF 通过对植物细胞造成损伤，来刺激植物机体分泌代谢产物

从而达到增加营养物质的积累。PEF 技术相对于传统的提取技术有具有更强的提取效率，这样使 PEF 对植物类营养物质提取量达到最大化。PEF 提取出来的营养物质对人体的保健作用更加明显。通过 PEF 刺激后生成的顺式异构体代谢物能够更好的被人体吸收，PEF 的提取物能显著提高细胞活力。我们可以通过这一理论为那些保健作用效果强但是含量较低的营养物质定制提取方法。

除了能加大生物活性物质积累，PEF 刺激后产生氧化应激损伤同样能达到杀灭微生物作用。膜损伤是微生物失活的主要原因，此外 PEF 还会通过氧化应激途径影响微生物的代谢，通过影响微生物的蛋白表达、损伤遗传物质来达到杀灭微生物的目的。在杀灭微生物的过程中没有热效应，不会影响食品的口感，甚至还会增加营养物质的积累。

通过 PEF 处理导致食品中营养物质含量增高，微生物含量低，食品质量参数如口感、颜色等没有不良反应，这样生产出来的食品可以在市场上作为商品或者食品加工原料。

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