纳米乳液提升植物精油的抗菌性能及其在食品表面的消毒作用研究进展

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摘 要: 食品及其加工设备和环境中的腐败菌和致病菌污染是影响食品质量安全的重要因素, 高效防控这些有害微生物在食品工业中具有重大需求。植物精油不仅具有快速杀菌的作用, 而且不易引发有害微生物对食品加工理化因子产生抗性。然而, 植物精油具有亲脂性和易挥发性等缺点, 限制了其在食品消毒领域的应用。纳米乳液技术可以将植物精油包埋在食品级表面活性剂中, 形成稳定性良好的分散体系, 从而较好地克服植物精油在食品工业中应用的瓶颈问题。因此, 本文综述了植物精油纳米乳液的制备方法、纳米乳液提升植物精油体外和体内抗菌活性的效果、纳米乳液在食品表面的抗菌膜作用, 以期为植物精油纳米乳液用于促进食品质量安全提供不可或缺的依据。

关键词: 纳米乳液; 植物精油; 抗菌; 抗菌膜

Research progress in antibacterial activity of essential oils enhanced by nanoemulsions and their application in food surface disinfection

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ABSTRACT: Contamination of spoilage and pathogenic microorganisms on food surfaces, food processing equipment and environment is an important factor affecting food quality and safety. Therefore, effective control of these harmful microorganisms is in great demand in food industries. It has been demonstrated that plant essential oils not only have a rapid bactericidal effect, but also are not easy to induce microbial resistance to food processing and storage-related stresses. However, the lipophilic and volatile properties of PEOs limit their applications in disinfection of food products. Nanoemulsion technology emerges as a potential technique to entrap PEOs in the food

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grade surfactants with enhanced diffusion to target food systems, which contributes to solving the bottleneck problems limiting the food application of PEOs. This review summarized the development methods and the improvements in antibacterial activities of PEO nanoemulsions *in vitro* and *in vivo*, furthermore, discussed their applications in food surface disinfection as an antibiofilm agent, so as to provide a theoretical basis for promoting the utilization of PEO nanoemulsions in food quality and safety assurance.

KEY WORDS: nanoemulsion; essential oil; antibacterial; antibiofilm

0 引　言

随着全球食品市场的发展，食物链更加广泛和复杂，食品安全问题越来越受到重视[1]。细菌、真菌及其毒素，不仅污染各类食品带来安全问题，还会造成食品品质和营养损失[2]。在食品工业中，卫生措施一般分为清洗和消毒两部分。消除食品和设备表面的有害微生物，降低其传播速度和范围，是保障食品质量安全的重要途径之一。目前广泛应用于食品行业的化学消毒剂，主要包括氧化剂、表面活性剂和醇类[3]。化学消毒剂具有一些潜在的危害，比如次氯酸盐会腐蚀设备表面并污染环境，长期使用可能对操作人员的健康造成影响。季铵盐类清洁剂虽然具有较高的安全性，但是消毒效果不如次氯酸盐[4]；当消毒效果不佳时，残留的微生物会在设备或食品表面形成菌膜，不仅难以被清除，而且极大提高了菌体抗性，进而威胁食品质量安全[5]。另外，一些化学物质如乙醇会诱导微生物产生抗性，降低消毒效果[6]。因此，亟待研发环境生物友好型的化学消毒剂替代品。

植物精油是提取自芳香植物的次级代谢产物，富含酚类和脂族化合物，具有独特的风味和广谱的抑菌活性，且对环境没有潜在的危害，因而其市场需求日益增长[7–8]。然而，植物精油具有亲脂性，难分散于水相，其稳定性受温度、光照等环境因素的影响较大，在食品领域的应用受到了诸多限制[9]。纳米包埋技术可将精油封装在合适的生物聚合物中，起到保护活性功效成分的作用，从而拓展植物精油的应用范围[10]。现有精油及其主要成分的纳米包埋技术中，以乳化剂为基础的递送体系广受关注，如纳米乳液、微乳液等[11–12]。其中，纳米乳液可利用少剂量的食品级表面活性剂，在一定的外部条件下，以纳米级液滴的形式高度分散于不相溶的另一相中，胶体分散体系的油水界面张力很低，极具实用价值[13]。

随着植物精油递送体系研究的不断深入，已有关于制备精油微乳液、微胶囊和脂质体等递送体系的综述，其对肉制品和果蔬的防腐保鲜作用已被大量研究证实[14–15]。此外，越来越多研究者开始关注植物精油对细菌菌膜的抑制和清除作用，以及在食品及食品设备表面的快速杀菌效果。然而，纳米乳液技术提高植物精油消毒杀菌作用还缺乏系统总结分析。基于此，本文首先介绍了植物精油的快速杀菌作用，其次阐述了植物精油纳米乳液的制备方法和纳米乳液技术提高精油抗菌作用的研究进展，重点总结了其在食品表面杀菌和抑制菌膜形成中的应用，以期为精油在食品表面和设备中的杀菌消毒应用研究提供参考依据。

1 植物精油的快速杀菌作用

根据已有研究，大多数提取自香辛料的植物精油对常见的食源性细菌具有较强的抑菌活性，包括大肠杆菌、金黄色葡萄球菌、枯草芽孢杆菌、沙门氏菌、单增李斯特菌和荧光假单胞菌等[16]。此外，植物精油不仅绿色安全，且不易诱导有害微生物产生抗性，是次氯酸钠和乙醇等传统化学消毒剂的潜在替代品[17–18]。

植物精油的杀菌性能一般取决于精油的成分和试验菌株。植物精油中含量最多的成分为萜类化合物，其次为带有苯环的酚类化合物[19]，一些植物精油在体外试验中具有良好的快速杀菌效果，可以在 30 min 内快速杀死多种食源性细菌[20]。例如，CAMPANA 等[21]报道 1%的香芹酚可以在 15 min 内，将 304 不锈钢表面形成的大肠杆菌 O157:H7、金黄色葡萄球菌、绿脓假单胞菌和粪肠球菌菌膜消除 5~7.5 log CFU/cm^2。此外，崔海英等[22]发现 0.05%的丁香精油对单增李斯特菌展现了较好的抗菌性能，在 8 h 内能杀死 99.996%的受试细菌。

在食品中应用时，SINGH 等[23]将百里香精油用于生菜和胡萝卜净菜加工，发现 1.0 mL/L 的百里香精油可以在 5 min 内杀死约 2 log CFU/g 数量的大肠杆菌 O157:H7。OLIVEIRA 等[24]发现以手动喷雾方式将 0.39%的丁香精油用于鸡蛋并干燥 30~50 min 后，可以达到多聚甲醛在鸡蛋表面的消毒效果。林琳等[25]发现肉桂精油对大肠杆菌 O157:H7 菌膜具有良好的清除作用，且 4.0 mg/mL 质量浓度处理可有效清除生菜等 5 种蔬菜表面残留的菌膜。总体而言，香辛料来源的植物精油在食品及食品加工设备表面均展示出了较好的快速杀菌性能。

植物精油在实际消杀应用时也存在诸多缺点，比如需要较高的使用浓度，才能达到商业化需要的杀菌效果[26]。植物精油的使用成本明显高于化学消毒剂，其挥发性气味也会带来负面影响。VALERIANO 等[27]报道将薄荷精油或香茅草精油和氢氧化钠配制成消毒溶液作用 20 min，可几乎完全清除 304 不锈钢表面的肠炎沙门氏菌菌膜。但是氢氧

化钠具有一定的腐蚀性，不能直接在食品表面使用。另外，食品中的蛋白质或者脂质成分，也会与植物精油互作，降低精油的杀菌活性[28]。因此，亟需研发纳米乳液等食品运载体系，扩大植物精油在食品工业中的应用范围。

2　植物精油纳米乳液的制备技术

植物精油纳米乳液的组成一般包括水相、油相和表面活性剂。根据组分和内外相分布不同，纳米乳液可分为水包油型(O/W)和油包水型(W/O)的双相乳液以及水包油包水型(W/O/W)和油包水包油型(O/W/O)的多相乳液[29]。应用于食品工业的植物精油纳米乳液通常为 O/W 型，这是一种热力学不稳定/动力学稳定的胶体分散系统[30–31]。由于油相和水相互不相溶，因此亲水性较强的乳化剂(如制备植物精油纳米乳液常用的吐温)可以降低水油界面张力，易形成 O/W 型纳米乳液[32]。

O/W 型植物精油纳米乳液一般通过两步形成，首先制备粗乳液，然后将其转化为纳米乳液。常见的制备技术分为低能乳化法和高能乳化法两种。制备方法的不同会影响纳米乳液的粒径、稳定性和表面活性剂的用量，其中，表面活性剂的添加范围通常在 5%~10%之间[33]。

2.1　低能乳化法

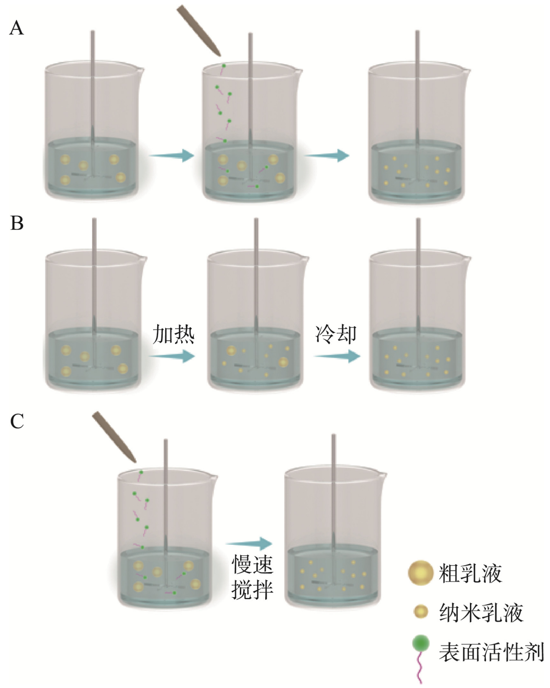
低能乳化法是在体系成分和环境条件发生某种变化时，乳液会自发形成纳米液滴[34]。常用的低能乳化法包括自发乳化、相转变温度法和相转变组分法[35–36]。

在自发乳化中，纳米乳液是在不利用机械力或温度变化的情况下而形成的。该方法通过添加一种表面活性剂来形成反转相[37–38](图 1A)。LIEW 等[39]用自发乳化法制备酸橙精油纳米乳液，将油相和水相以 2:8 (V:V)的比例混合来制备酸橙纳米乳液，油相由 5%的油(酸橙精油+玉米油)和 15%的表面活性剂吐温 80 组成，水相由 80%的蒸馏水组成，得到纳米液滴的粒径最低为 21 nm。

相转变温度法制备纳米乳液分 3 个步骤(图 1B)。首先，将表面活性剂、油相和水相在室温下混合搅拌，制备表面活性剂油水乳液。然后，加热表面活性剂油水乳液。当形成纳米乳液时，在同时搅拌的情况下快速冷却或用冷水稀释以形成 O/W 纳米乳液[40–41]。TUBTIMSRI 等[42]通过此法将由薄荷油、椰子油和表面活性剂组成的油相加热至 62℃，随后加入 65℃的水相中，在 3800 r/min 下均化 5 min，最后冷却至室温制备得到纳米乳液，获得最小的液滴尺寸(55.8 nm)。同样，CHUESIANG 等[43]在制备肉桂精油纳米乳液时，使用了 15%和 20%的吐温 80，将肉桂精油和中链甘油三酯混合 3 min 后进行两步冷却过程，得到的纳米液滴粒径分别为 50.70 和 23.50 nm。

相转变组分法是改变体系中各组分比例，从而发生相转变(图 1C)。相转变组分法中影响乳液粒径的其他因素有乳化过程中水相滴加速度、搅拌的充分程度等环境因素[44]。任婧楠等[45]采用相转变组分法制备了甜橙精油纳米乳液和 D-柠檬烯纳米乳液，使用了 6%~20%的吐温 80，得到的乳滴粒径大多分布在 8.51~11.65 nm。

总体而言，低能乳化法不需要特殊的设备，制备工艺简单，易获得分布窄和粒径小的植物精油纳米乳液，但是表面活性剂添加量通常高于 10%[46]。



注：A 自发乳化法；B 相转变温度法；C 相转变组分法。

图 1　3 种低能乳化法

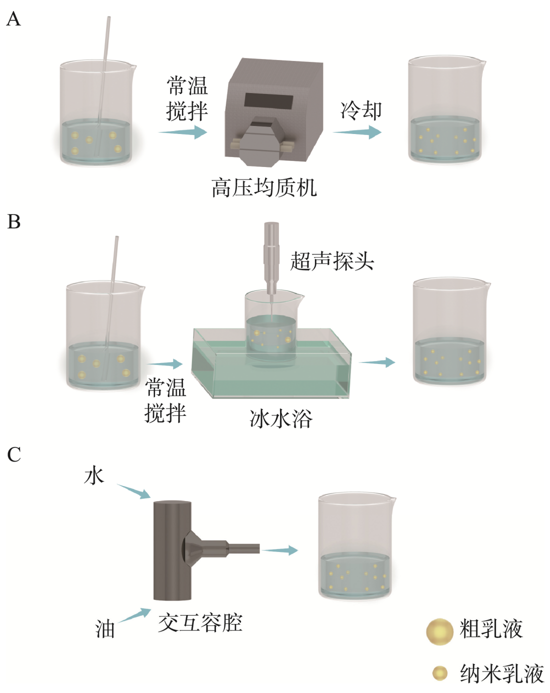
Fig.1　Three types of low-energy emulsification methods

2.2　高能乳化法

高能乳化法一般是指通过高压均质法、超声乳化法和高压微射法等需要大量能量的乳化方法，将普通乳液的大液滴进行拉伸破碎，使大液滴分散为数个小液滴，从而制得粒径在纳米级别的乳液[47]。高压均质法是在非常高的压力下，迫使粗乳液通过一个小阀门，产生强烈的剪切力、空化力和湍流力，从而减小分散在水相中的油颗粒的尺寸[48–49](图 2A)。超声乳化法的作用机制与高压均质相似，是由高强度超声波产生空化泡，空化泡持续增长会产生内爆，引起喷射流，对分散的大液滴施压，使其转化为小液滴[50](图 2B)。高压微射流技术是指乳液在高压(100~800 MPa)下通过具有固定几何形状的交互容腔，两股粗乳化液从两个相对的通道中以高速状态相互加速碰撞，产生巨大的剪切作用力而形成的[51](图 2C)。

YANG 等[52]采用超声乳化法制备了一种新型的柠檬醛纳米乳液，得到的乳液液滴粒径平均值在 29.4 nm。史亚

濛[53]使用高压均质法制备了花椒精油纳米乳液，其中最小粒径为 20.97 nm。彭群等[54]采用高压微射流法制备了橙皮精油纳米乳液，粒径基本都达到 200 nm 以下。高能乳化法通常不需要添加稳定剂、增稠剂、助溶剂和助表面活性剂[55]。目前应用最多的高能乳化法是超声法和高压均质法，与低能法相比其制备成本较高。另外，使用超声法制备纳米乳液的过程中会产生大量的热，因此通常需要在冰浴条件下对精油这类易挥发的芯材进行超声[56]。



注: A 高压均质法; B 超声乳化法; C 高压微射法。

图 2 3 种高能乳化法

Fig.2 Three types of high-energy emulsification methods

3 纳米乳液对植物精油抗菌活性的改善效果

3.1 体外抑菌作用

纳米乳液的粒径一般小于 200 nm，因此能更好的分散于水相，使精油更快地传输到微生物细胞膜并与之相互作用，增加了接触表面积，以此来提高植物精油的抗菌活性[57–60]。

已有大量体外研究表明，纳米乳液可以提高植物精油对常见食源性细菌的抑菌活性。ÖZOGUL 等[61]通过抑菌圈试验揭示月桂精油纳米乳液对金黄色葡萄球菌和粪肠球菌的抑菌活性高于游离精油。ALMADIY 等[62]测定抑菌圈、最低抑菌浓度(minimal inhibitory concentration, MIC)和最低杀菌浓度(minimum bactericidal concentration, MBC)，发现纳米乳化提高了蓍草类精油 *Achillea biebersteinii* 对金黄色葡萄球菌的抑菌活性。GHOLAMHOSSEINPOUR 等[63]构建了一款食品级的香薄荷属精油 *Satureja sahendica* 纳米乳液，其对金黄色葡萄球菌、枯草芽孢杆菌、宋内志贺氏菌、伤寒沙门氏菌和大肠杆菌的抑菌活性均显著高于游离精油。SEPAHVAND 等[64]发现百里香精油对金黄色葡萄球菌、大肠杆菌和产气荚膜杆菌的 MIC 值大约是其纳米乳液剂型的 2 倍。徐冰等[65]对百里香纳米乳液进行研究发现，游离百里香精油对大肠杆菌的 MIC 值和 MBC 值分别是纳米剂型的 4 倍和 8 倍，对金黄色葡萄球菌的 MIC 值和 MBC 值均是纳米剂型的 4 倍。此外，纳米乳液也能提高植物精油对真菌的抑菌活性。LIU 等[66]对肉桂精油纳米乳液进行测试，发现其对黑曲霉的抑菌作用最高，MIC 值比游离肉桂精油降低了一半。LIANG 等[67]发现薄荷精油纳米乳化前后对金黄色葡萄球菌和单增李斯特菌的 MIC 值没有显著变化；但是在动态杀菌试验中，纳米乳液比游离薄荷精油杀菌效果更强，起到了提高快速抗菌活性的效果。

纳米乳液技术可以提高精油体外抑菌效果的机制，与细胞膜的通透性相关。纳米乳液技术降低了精油油滴的粒径，增加了精油活性成分与细胞表面的接触面积，加快了细胞表面形态的变化和细胞内容物的泄露[68–69]。但是，也有一些植物精油在纳米乳化后，体外抑菌活性低于游离精油，目前关于纳米乳化包埋技术对植物精油抑菌活性的影响规律尚缺乏较为系统的研究[70–71]。

3.2 果蔬表面杀菌作用

鲜切果蔬在加工过程中要经过清洗、消毒、去皮和切片等工序，易受单增李斯特菌和沙门氏菌等细菌的污染[72–73]。传统的含氯消毒剂可能与鲜切果蔬成分中的有机物质发生反应，生成三卤甲烷和氯胺[74]。因此，研发以植物精油为主要成分的果蔬消毒剂(如精油纳米乳液)广受关注。虽然植物精油纳米乳液可以作为可食性涂层的抑菌成分，但是直接在鲜切果蔬上使用，精油的气味会对食品感官品质产生一定影响[75]。

植物精油纳米乳液代替化学消毒剂用于鲜切果蔬的清洗过程，不仅起到快速杀菌的作用，也避免了精油气味对食品感官品质的影响，扩大了精油纳米乳液在净菜加工领域的应用范围。DÁVILA-RODRÍGUEZ 等[76]将牛至精油纳米乳液应用于接种了大肠杆菌和单增李斯特菌的新鲜芹菜上，发现 2 种受试细菌的数量在 60 min 内减少了 5 log CFU/g。RU 等[77]研究了月桂精油纳米乳液对鲜切甜瓜微生物特征的影响，发现经过纳米乳液处理 8 d 后的甜瓜与对照组相比需氧菌数量减少了 4.28 log CFU/g，霉菌和酵母减少了 2.12 log CFU/g。PAUDEL 等[78]使用 0.5%的肉桂油纳米乳液对接种了单增李斯特菌和沙门氏菌的甜瓜进行杀菌试验，发现与对照组相比经过纳米乳液处理过的甜瓜中 2 种致病菌分别减少了 7.7 和 5.5 log CFU/g，证明了肉桂油纳米乳液具有减少甜瓜等新鲜农产品中重要致病菌的潜力。LUCIANO 等[79]用 0.3 和 0.15 μL/mL 柠檬醛纳米乳液处理接种了单增李斯特菌的鲜切甜瓜和木瓜，发现

在 4℃下储存 48 和 72 h 后 2 种样品中的微生物数量均低于 1 log CFU/g。此外，植物精油纳米乳液也适合与其他方法联合使用。YANG 等[80]研究了超声波联合柠檬醛纳米乳液对紫甘蓝表面微生物的消减效果；结果表明，0.3 mg/mL 柠檬醛纳米乳液在超声联合杀菌 8 min 后，导致鼠伤寒沙门氏菌数量减少了 9.05 log CFU/g。

由此可知，植物精油纳米乳液对果蔬中的重要致病菌具有较好的消减效果，而且其可与其他控菌方法联合使用，以便更好地用于食品的清洗消毒。

4　纳米乳液对植物精油清除细菌菌膜作用的改善效果

食源性腐败和致病微生物形成的菌膜，对食品质量安全而言是一个重要危害因子[81]。菌膜状态下的微生物能够附着和生长在食品表面和其他加工设备表面(主要是不锈钢材质)，从而附着在食品、食品加工设备和环境，导致食品持续性污染[82–83]。此外，菌膜中的扩散屏障可阻止外界抗菌物质的渗入，增加了对菌膜清除的难度[84]。多项研究表明，与浮游态菌体相比，菌膜对干燥、紫外线辐射以及抗生素、表面活性剂、消毒剂等抗菌剂的抵抗力更强[85–88]。

植物精油可以干扰菌膜的形成过程，油相的亲水性部分可以穿透菌膜的胞外多糖，而疏水性部分能够渗透细菌膜的脂质成分[89]。植物精油通常需要较高的使用浓度才能抑制菌膜，纳米乳液技术极大的改善了精油抑制菌膜这一应用的瓶颈。GHADERI 等[90]发现香薄荷属精油 *Satureja khuzistanica* 纳米乳液在 4 mg/mL 质量浓度下显示出最佳的抗铜绿假单胞菌菌膜活性，达到接近 90%的抑制率和超过 50%的清除率。DA-SILVA 等[91]对卡图巴精油纳米乳液进行研究发现，使用质量浓度为 8 mg/mL 时对单增李斯特菌菌膜形成的抑制率为 58.9%。HASSANSHAHIAN 等[92]报道骆驼刺精油纳米乳液对鲍曼不动杆菌和大肠杆菌菌膜的最低清除质量浓度分别为 12.5 mg/mL 和 1.75 mg/mL。

当新鲜果蔬相关的有害微生物以菌膜的形式存在时，它们的危害更大。沙门氏菌、李斯特菌、葡萄球菌和大肠杆菌在内的不同致病菌在食品表面黏附，随后形成菌膜，可导致食品变质或疾病传播[93–94]。PRAKASH 等[95]用芳樟醇纳米乳液对鼠伤寒沙门氏菌菌膜进行研究，发现与芳樟醇相比其纳米乳液能更有效地杀死鲜切菠萝表面的细菌，这种增强功效可能由于纳米乳液更易于穿透细胞膜。AMRUTHA 等[96]也报道孜然芹精油纳米乳液对新鲜水果和蔬菜源的大肠杆菌和沙门氏菌菌膜的抑制率分别为 42.56%和 38.92%。LI 等[97]用百里酚纳米乳液对生菜和蓝莓表面的食源性细菌菌膜进行消毒，发现在室温下洗涤 60 s 后，大肠杆菌 O157:H7、单增李斯特菌和鼠伤寒沙门氏菌菌膜中的细菌数量降低了约 2 个数量级。

因此，纳米乳液对果蔬表面多种有害微生物的菌膜均具有较好的清除作用，而且部分剂型的效果优于游离精油。然而，目前罕有植物精油纳米乳液对食品表面混菌菌膜作用效果的报道，在未来研究中可予以重点关注，以获得更贴近食品生产实际的数据。

5　结束语

随着消费者对天然抗菌物质及相关产品的需求与日俱增，植物精油以其独特的香气和优良的抑菌活性等优点备受关注。在众多纳米装载体系中，纳米乳液利用表面活性剂为基质包埋芯材，克服了精油的挥发性和疏水性等缺点，并提高其在食品加工因子(如温度、pH)作用下的稳定性，促进精油更有效地用于食品卫生和防腐保鲜等方面。

目前关于植物精油纳米乳液消毒作用的研究并不全面，尚存在以下挑战：(1)植物精油纳米乳液的配方应根据应用场景合理研发，实现更高效的快速杀菌作用；(2)纳米乳化如何提高植物精油的杀菌和抗菌膜效果及其机制研究相对缺乏，在一定程度上限制了植物精油纳米乳液在不同食品和设备上的精准使用；(3)应该更全面地评估植物精油纳米乳液的使用成本问题。总之，植物精油纳米乳液可以开发成食品级安全的新型消毒剂，减少化学消毒剂使用带来的潜在健康危害，促进食品行业的绿色发展。

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